Retinopathy and Nephropathy in Diabetes Mellitus

Comparison of the Effects of Two Forms of Treatment

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The introduction of insulin in the treatment of diabetes mellitus about thirty-five years ago marked a radical change in the prognosis of the disease, especially in young patients. Diabetic coma, once so often fatal in young patients, is now fairly seldom the cause of death. But this increase in the duration of survival of diabetics has gradually given rise to new and serious problems, the so-called late complications of diabetes. The lesions of the capillaries and venules in the kidney and the eye have been regarded as so characteristic as to be almost pathognomonic. Although the alterations in the arteries and arterioles in diabetics are not histologically specific, some investigators[2] claim that all the vascular lesions in diabetics represent a specific vascular disease, angiopathia diabetica, responsible for all of the late vascular manifestations of diabetes.

Most interest has been focused on retinopathy and nephropathy in young diabetics. These complications have been found to be common after diabetes of more than ten years' duration (table 1).

In Fanconi's[10] series of juvenile diabetics all of the patients who had had diabetes for sixteen years or more also had nephropathy, and none lived more than twenty-one years after the onset of diabetes.

It is apparent from earlier investigations that the development of these complications is dependent mainly on the duration of diabetes. Sex, severity of diabetes and age at onset, on the other hand, appear to be of minor importance. Whether control of diabetes per se is of any significance in this respect is still debatable.

Adherents[11–15] of "free diet" claim that dietary restrictions will have but little influence on the development of late complications, while other investigators[3–7,9,12,16–28] share Joslin's view that, although proper control of the diabetes will not prevent late manifestations of diabetes, it will at least delay them.

The first to show that the frequency of retinopathy and nephropathy was significantly higher in poorly controlled diabetics were Keiding, Root and Marble[23] (tables 2 and 3).

The literature contains only one investigation in which the patients had always been on a free diet and were afterwards thoroughly examined. This study (Englesson 1954) showed that of 107 patients who had developed diabetes before the age of forty, only 6.5 per cent were free of vascular complications after more than ten years of diabetes.

Englesson's gloomy results contrast strikingly with the brighter outlook suggested by the observations of Sherrill[25] in patients selected because of long duration of diabetes and their outstanding fidelity, patience and perseverance in adhering to dietary programs. They were also selected because most of them had come under Sherrill's supervision early in the disease. The material consisted of thirty-four patients who had developed diabetes before the age of forty and who had had the disease for twenty to thirty-eight years (average 26.5 years). Of these, only four (12 per cent) showed signs of nephropathy. The ocular fundi were of normal appearance in five (15 per cent) patients; they showed a few single microaneurysms in twenty-two (65 per cent) and retinopathy grade II in the remaining seven (20 per cent).

Inasmuch as Sherrill's material was selected, it teaches nothing about the over-all frequency of retinopathy and nephropathy, but suggests that proper control of diabetes can delay development of these complications.

BACKGROUND AND PURPOSE OF THE STUDY

The present investigation was prompted by the observation that some of our patients who had had diabetes for more than twenty-five years showed at most slight late complications. We therefore considered it worth while to review all of the diabetics in Malmö in whom the disease had been diagnosed between the years 1922 and 1935 and who were less than forty years of age at onset of the disease. In the beginning of their diabetes all of these patients had been on a strictly regulated diet and attempts had been made to

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TABLE 1

Influence of duration of diabetes on the development of retinopathy and nephropathy

Author	Age at onset of diabetes	Number of patients	Per cent incidence of retinopathy (R) and nephropathy (N) according to the duration of diabetes in years					
			10-15		15-20		20-25	
			R	N	R	N	R	N
Bartels and Poulsen[3]	<16	116			27	27*		
Englesson[4]	<40	107	71	81.4†				
Jackson et al.[5]	<16	75	47	4‡				
Kerr et al.[6]	<16	81	37	0	69	19	75	54
Lundbaek[7]	<40	89			76	18	87	23
Skouby[8]	<40	137	47	5	70	23	70	40
White and Waskow[9]	<16	350	10	5	45	30	80	50

*15-31 years' duration. †10-25 years' duration. ‡>10 years' duration.

TABLE 2

Correlation of incidence of retinopathy with degree of control and duration of diabetes (Keiding, Root and Marble)

Duration of diabetes (years)	10-15		15-20		>20	
	Number of patients	Per cent retinopathy	Number of patients	Per cent retinopathy	Number of patients	Per cent retinopathy
Good control	17	35 (7)	12	60 (25)	32	55 (25)
Fair control	29	40 (17)	22	65 (50)	41	70 (50)
Poor control	108	70 (46)	74	78 (64)	116	82 (70)

(Values in parentheses denote all but minimal lesions.)

TABLE 3

Correlation of incidence of nephropathy with degree of control (Keiding, Root and Marble)

	Duration 10-36 years	
	Number of patients	Per cent
Good control	61	2
Fair control	92	17
Poor control	298	28

keep the urine as sugar-free as possible. During the latter part of the 1930's, a more liberal diet was allowed and soon afterward the so-called free diet became the rule. The importance of glycosuria was belittled, and as long as the patients had no polyuria or ketonuria, their condition was regarded as satisfactory. Therefore, in an attempt to form an opinion of any influence of proper control of the diabetes on the late complications, diabetics in whom the disease had been discovered from 1936 to 1945 were also reviewed. These two series thus include all cases of diabetes discovered between 1922 and 1945 in Malmö inhabitants less than forty years of age at the time of the diagnosis. All were reviewed for nephropathy and retinopathy.

MATERIAL

All diabetics in Malmö had been cared for at the same hospital where the inauguration and principles of treatment in the beginning of the disease were uniform. According to the public census, Malmö had 113,602 inhabitants in 1922, which increased to 171,053 in 1945.

The two series are compared in table 4.

The patients who are not included in analysis have not survived long enough to permit evaluation of the effect of treatment on late manifestations (seventeen patients in Series I; ten in Series II). All except three of these twenty-seven patients died before they had had diabetes for five years and all had no proteinuria at the time of death.

The series did not differ much in age at onset of the disease.

Of the nine patients in Series I who had died in diabetic coma, death occurred in three before insulin had become available and three during the first year after the introduction of insulin.

Of the nineteen patients in Series I who had died from late complications of diabetes, death was due to typical Kimmelstiel-Wilson syndrome after an average duration of diabetes of twenty-two years (seventeen to twenty-nine years) in eight, while eleven patients died from cardiovascular complications at an average age of forty-nine years and after an average duration of diabetes of twenty-one years (fifteen to thirty years).

In Series II, four patients had died from Kimmelstiel-

TABLE 4
Comparison between the two series at review

	Series I (1922-1935)	Series II (1936-1945)
Average age at onset (years)	20.9	19.3
Number of patients	71	115
Not included in analysis	17	10
death from:		
diabetic coma	9	4
pulmonary tuberculosis	4	2
other diseases	4	4
Remainder	54	105
Deaths from late diabetic manifestations	19	5
Kimmelstiel-Wilson	8	4
Cardiovascular lesions	11	1
Still alive	35	100
Reviewed	35	99

Wilson syndrome after an average duration of the diabetes of sixteen years and one from coronary thrombosis.

This thus left thirty-five patients in Series I and 100 in Series II. All of the patients were examined except one in Series II.

NEPHROPATHY

When proteinuria had been found in more than half of all examinations during the last year, the patient was said to have nephropathy. The frequency of nephropathy in each series is given in table 5. These series include the living patients as well as those who died from late complications before the time of the review.

Series I includes two additional patients, who died from pulmonary tuberculosis and pneumonia, respectively, after having had diabetes for nineteen years. They were not included in the original material because the ocular fundi had not been examined.

TABLE 5
Incidence of nephropathy in the two series including living and fatal cases

	Series I	Series II
Duration of diabetes in years	19-35 (24.5)	10-21 (15.9)
Number of patients	56	104
Patients with nephropathy	18 (32%)	56 (54%)

The frequency of nephropathy was lower in Series I (32 per cent against 54 per cent) though the patients in Series I had had diabetes for 8.6 years longer than those in Series II. The difference is statistically significant ($p<0.001$).*

In order to compare the frequency of nephropathy in the two series after diabetes of equally long duration (fifteen years), all patients who had had diabetes for at least fifteen years were examined to determine if they had proteinuria after that time. Those patients who died before they had had diabetes for fifteen years and had proteinuria were assigned to the group with nephropathy. The results are given in table 6.

TABLE 6
Incidence of nephropathy in the two series after fifteen years' duration of diabetes

	Series I	Series II
Number of patients	56	57
Patients with nephropathy	5 (9%)	35 (61%)

After equal duration of diabetes (fifteen years) the incidence of nephropathy in Series II was seven times as high as in Series I.

RETINOPATHY

The frequency of retinopathy could be judged only in those patients living at the time of the review, because several of those patients who had died earlier had not been studied ophthalmologically during the last years of their lives.

Since the number of patients who died from Kimmelstiel-Wilson's syndrome and who almost have retinopathy was larger in Series I than in Series II, more importance must be attached to the renal changes than the ophthalmologic findings at the review.

Of the patients belonging to Series I and still living, two could not be included in this part of the investigation; one of them was not examined ophthalmologically and in the other who had been operated on for bilateral cataract, the appearance of the retina could not be judged with certainty. Of the patients in Series II, two have not been examined for retinopathy. All of the examinations were carried out by accredited ophthalmologists.*

Retinopathy was recorded in four degrees according to Scott.[21]

I. Only few microaneurysms and no exudates seen.

II. Several microaneurysms and a few to many small white exudates.

III. Large hemorrhages, many white exudates and several microaneurysms.

IV. Severe advanced retinopathy of proliferative type, retinal detachment and acute or chronic hemorrhagic glaucoma.

The frequency and the degree of retinopathy in the two series is given in table 7.

Retinopathy was seen in 49 per cent of the patients in Series II, as against 58 per cent in Series I. The

*The x^2 method was used in the statistical treatment of the data.

*I beg to express my thanks to Drs. Ake Arén, Magnus Blix and Claes Haptén for ophthalmoscopic examination of the patients.

TABLE 7
Incidence of retinopathy in the two series (only living patients)

	Series I	Series II
Duration of diabetes in years	21-35 (26.5)	10-21 (15.9)
Number of patients	33	97
Normal ocular fundi	14 (42%)	49 (51%)
Retinopathy degree I	13 (40%)	16 (16%)
II	5 (15%)	14 (14%)
III	— } (3%)	9 } (19%)
IV	1 }	9 }

difference was not significant. Severe retinopathy (degrees III and IV), on the other hand, was significantly ($p<0.01$) more common in Series II (19 per cent against 3 per cent) despite the fact that the duration of diabetes was more than ten years shorter than in Series I.

In Series I 85 per cent had normal vision on both sides as against 75 per cent in Series II. This difference was not statistically significant. Only persons with a visual acuity of at least 0.7 in one eye and 0.3 in the other, or at least 0.8 in one eye, can be granted a driving license in Sweden. In only one patient (3 per cent) in Series I was vision insufficient for granting of a driving license, as against twelve (13 per cent) in Series II. The difference was significant ($p<0.05$). Of Series II, five patients are blind or practically blind, while the patient with the poorest vision in Series I had a visual acuity of 0.3 on both sides.

To sum up, of fifty-four patients in Series I, who could be judged regarding frequency of late complications and had had diabetes before the age of forty, about two thirds were still living after having had the disease for an average of 26.5 years (range twenty-one to thirty-five years). All claimed that their working capacity had not been impaired by the disease and none had had myocardial infarction, cerebrovascular complications or gangrene, despite the fact that the mean age at the time of the review was forty-nine years. Only 14 per cent had proteinuria; in somewhat more than 40 per cent the ocular fundi were found to be normal (none of these patients had proteinuria), and an additional 40 per cent had only scanty microaneurysms. Only 15 per cent had decreased vision, and in all except one of these vision was sufficient to obtain a driving license.

PRINCIPLES OF TREATMENT OF DIABETES IN THE TWO SERIES

Any attempt to form an opinion in retrospect of the severity of hyperglycemia and glycosuria during all these years simply on the basis of a few annual determinations of the blood and urinary sugar might appear meaningless. However, as mentioned, the two series differ in the principles of treatment of the diabetes. It was therefore decided to analyze the series for differences in diet, insulin treatment, frequency of diabetic and insulin coma, insulin reactions and body weight.

The principles of treatment of diabetes during the years 1922 to 1935, i.e., the period covering the time of onset in Series I, in Malmö was as follows: The patients were given a low-calorie diet consisting of plenty of low-carbohydrate vegetables, very little bread and potatoes, protein-rich foodstuffs and a moderate amount of fat. If this diet failed to render the urine sugar-free, treatment with three daily injections of Regular Insulin was started. Serious attempts were made to control the glycosuria completely. During as well as after the period of regulation in the hospital everything was done to compose a suitable diet and adjust the insulin requirements as exactly as possible. Broadly speaking, the dietary restrictions were almost as stringent as during the pre-insulin era. All of the patients in Series I were thus informed from the very beginning that maintenance of comfort and vigor depended upon careful observance of the dietary prescriptions.

During the latter part of the nineteen thirties more liberal dietary regimens became increasingly popular and about 1940 marked the beginning of the "free diet." The only dietary restriction recommended was the avoidance of sugar. If the patient was free from ketonuria, excessive thirst or polyuria at follow-up, management of diabetes was considered satisfactory.

DIET

All patients were asked how long they had lived on a strictly regulated diet. In series I the patients had been on a strict diabetic diet for the first ten years on the average; in Series II, for about 2.5 years. In Series II forty-six of the ninety-nine patients had been on a free diet the whole time; the entire Series II had been on a free diet for a period corresponding on the average to 85 per cent of the total duration of diabetes. Some of the patients in Series I had indulged in "free diet" in recent years; the patients in Series I had been on a free diet for an average period corresponding to 18 per cent of the total duration of diabetes.

TREATMENT WITH INSULIN

All patients in both series had received insulin treatment, practically speaking, ever since onset of the disease. At the time of onset of the disease in Series I only short-acting insulin was available, and then the patients received two or three injections daily. At the

beginning of the period covered by Series II long-acting insulin was placed on the market and then it was possible to give the patient only one injection a day.

Of the thirty-five patients in Series I, eighteen (51 per cent) had two injections of insulin per day ever since onset of diabetes as against twenty-six (26 per cent) of the ninety-nine patients in Series II. In Series I the patients had one daily injection of insulin only during an average period corresponding to 10 per cent of the total duration of diabetes, the corresponding figure for series II being 45 per cent.

DIABETIC COMA

The frequency of diabetic coma in the series is given in table 8. The only difference between the two series with respect to the occurrence of diabetic coma was that seven of the patients in Series II, but none in Series I had had coma on three occasions or more. The percentage of patients who had never had diabetic coma was equal in both series.

TABLE 8

Incidence of diabetic coma

Attacks of diabetic coma	Series I (35 patients)	Series II (99 patients)
0	21 (60%)	60 (60%)
1	10 (29%)	26 (26%)
2	4 (11%)	6 (6%)
3		1 (1%)
4		3 (3%)
5		2 (2%)
6		1 (1%)
Average	0.5 per patient	0.7 per patient

INSULIN COMA

The frequency of insulin coma in the two series is given in table 9.

The number of patients who had never had insulin coma was lower in Series I, while the number who had had insulin coma on more than twenty occasions was higher in Series I. Both differences are statistically significant ($p<0.01$ and <0.001).

TABLE 9

Incidence of insulin coma

Attacks of insulin coma	Series I (35 patients)	Series II (99 patients)
0	12 (34%)	54 (54%)
1-4	10 (28%)	32 (32%)
5-9	4 (11%)	5 (5%)
10-19	1 (3%)	3 (3%)
20-50	6 } (23%)	4 } (5%)
>50	2 }	1 }

INSULIN REACTIONS

All of the patients were questioned as to how often they had had insulin reactions (table 10).

The two series differed significantly regarding the number of patients who had had insulin reactions on less than ten occasions per year ($p<0.05$) and of those who had had such reactions on more than thirty occasions per year ($p<0.001$).

TABLE 10

Incidence of insulin reactions

Number of insulin reactions per year	Series I (35 patients)	Series II (99 patients)
<10	11 (31%)	45 (45%)
10-30	11 (31%)	39 (39%)
30-50	3 } (37%)	9 } (15%)
>50	10 }	6 }

BODY WEIGHT

All patients were weighed at the time of review. The results published by Bøe et al.[35] on the basis of an investigation of the adult population in Bergen, Norway, were used in the calculation of normal weights. A deviation of ±5 per cent was allowed. One of the patients in Series I and three in Series II were not included in this analysis because their stature or age (below fifteen years) was above or below the range given by Bøe et al. The results are given in table 11.

TABLE 11

Body weight at the time of review

	Series I (34 patients)	Series II (96 patients)
Underweight	19 (56%)	40 (42%)
Normal weight	13 (38%)	35 (36%)
Overweight	2 (6%)	21 (23%)

The review showed that the average body weight in Series I was somewhat less than in Series II. In Series I average body weight was 8 per cent below normal as against 3 per cent below normal in Series II. The two series differed from one another statistically regarding the frequency of underweight as well as the frequency of overweight ($p<0.05$ and <0.01, respectively).

DISCUSSION

In earlier investigations of the influence of the control of the diabetes on the frequency of nephropathy and retinopathy the patients reviewed were practically always divided into so-called "good," "fair" and "poor" control. But any attempt to group a series in this way with the help only of a few annual office visits of pa-

tients who have had the disease for ten to thirty-five years must be extremely difficult, especially as it is known that some patients observe their diet very carefully a few days before examinations. Another objection against such classification is that some patients with good control may have diabetes of a type easier to control, possibly representing a form of diabetes in which complications appear later.

These sources of error can be obviated by comparing two groups of patients that have received treatment according to different principles.

As to the care of the patients, Series I differed considerably from Series II. The patients in Series I had been on a strictly regulated diet for four fifths of the time they had had diabetes. Series II had been allowed a free diet during 85 per cent of the total course of their diabetes. The percentage of patients who had used two or more injections of insulin a day was also higher in Series I than in Series II. As might be expected from the dietary regimen and the insulin requirements, insulin reactions were more common in Series I than in Series II.

These observations support the assumption that the hyperglycemia in Series I was less pronounced than in Series II.

The series also differed regarding retinopathy and especially nephropathy. Proteinuria was much less frequent in Series I though the patients had had their diabetes 8.5 years longer. In those patients who had had diabetes for fifteen years, nephropathy was seven times as common in Series II as in Series I.

The two series did not differ significantly in frequency of retinopathy. On the other hand, although the duration of diabetes in Series I was 10.5 years longer than in Series II, severe retinopathy was more common in the latter, as was the frequency of patients whose vision was too poor for them to be granted a driving license.

These results are, however, not so important as the figures found for nephropathy, because only the surviving patients could be examined.

In most series on record retinopathy antedated nephropathy and was also more common, as was the case in Series I. In Series II retinopathy and nephropathy were equally common. In Englesson's material,[4] which consisted only of patients on a free diet, nephropathy antedated retinopathy. It thus appears that poor control of the diabetes favors nephropathy. This complication is undoubtedly the more serious of the two. Thus Mann et al.,[26] White and Wascow[27] and Englesson[4] found the average survival after the appearance of nephropathy to be 3.5 to 6 years. Mild retinopathy, on the other hand, may remain stationary for several years.

The influence of the control of diabetes on the appearance of late complications could, of course, be assessed best in a series in which every other patient received a strictly regulated diet and the remainder a free diet. But no such material is available. Strictly speaking, Series I cannot be regarded as really well controlled because the patients had been on a free diet during 18 per cent of the course of diabetes, on the average. Neither was Series II a true "free diet" series, because those patients had been on a regulated diet for 15 per cent of the total period. Despite this, and despite the fact that the patients in Series I had had diabetes much longer than those in Series II, the frequency of nephropathy as well as that of severe retinopathy was statistically higher in Series II than in Series I.

These results support the increasingly common opinion that good control of diabetes delays the development of late diabetic manifestations. Possibly it is not the hyperglycemia per se that is responsible for the late complications of diabetes, but a hypothetical unknown responsible factor or factors which appears to run parallel with the degree of hyperglycemia. As long as the etiological factor remains unknown, the only thing we can do to delay late complications of diabetes is to try to control carbohydrate metabolism as carefully as possible. This is much more important than hitherto supposed by patients and doctors, especially concerning juvenile diabetes. It is surely important that young diabetics receive intense insulin treatment as soon as possible and that they are convinced from the very beginning that control of diabetes requires rigid dietary restriction with a constant carbohydrate intake from day to day. The calorie intake should be sufficient to permit normal growth and development of children, but after they have passed puberty, body weight should be kept somewhat below normal.

Patients with severe diabetes cannot control their disease with one dose of insulin daily, no matter whether they use Protamine Zinc, NPH or Lente insulin. Well-controlled diabetes with two daily injections of insulin is preferable to the less well-controlled diabetes which is the rule when only one injection daily is given.

The application of these principles of treatment aimed at keeping the urine as nearly sugar-free as possible naturally involves a greater risk of insulin reactions than does a more liberal regimen. Since insulin coma is accompanied by increased activity of the hypophysis-adrenocortical system, it has been suggested that insulin

coma might contribute to the late complications of diabetes. Observations in Series I, however, did not support this opinion. The eight patients who had had insulin coma on more than twenty occasions, had diabetes for 25.5 years on the average. None of them had proteinuria, four had normal ocular fundi, and the remaining four had only a few microaneurysms. All eight had normal vision on both sides. This suggests that insulin reactions per se do not appear to increase the risk of retinopathy or nephropathy.

SUMMARY

All patients in Malmö in whom diabetes was discovered during the years 1922-35 (seventy-one patients—Series I) and those in whom the disease was discovered between 1936 and 1945 (115 patients—Series II) and who were below forty years of age at that time were reviewed for the occurrence of nephropathy and retinopathy. After elimination of those patients who died before they had had diabetes long enough to develop late complications, there remained fifty-four and 105 patients, respectively. Nineteen of the patients in Series I and five in Series II had died from late diabetic complications. All patients except one in Series II were seen by the author personally.

The frequency of nephropathy in Series I was significantly lower even though the patients had had diabetes for 8.5 years longer than those in Series II. (These figures also include those patients who had died from late complications of diabetes.)

No significant difference was found in the frequency of retinopathy (difference in duration of 10.5 years), while severe retinopathy and severe impairment of vision were significantly more common in Series II, although the duration of diabetes was on the average ten years shorter than in Series I. (This analysis includes only patients who were alive at the time of the review.)

During the first period the patients were on a strict low carbohydrate and low calorie diet. Toward the end of the nineteen thirties dietary restrictions became much less rigid and soon afterwards the patients were placed on free diets. No attempts were then made to keep the glycosuria as low as possible. In Series I the patients were on a strictly controlled diet for ten years on the average and on a free diet during 18 per cent of the total course of diabetes. The patients of Series II had been on a regulated diet for 2.5 years and on free diet during 85 per cent of the total period of diabetes.

During an average period corresponding to 10 per cent of the diabetes period the patients of Series I received only one daily injection of insulin as against 45 per cent in Series II. Both the frequency of insulin coma and of insulin reactions were significantly higher in Series I. Body weight at the time of the review was significantly lower in Series I. The diet and the insulin treatment strongly suggest that the hyperglycemia was less in Series I than in Series II.

The review suggests that careful control of the diabetes delays the development of nephropathy and retinopathy.

The eight patients in Series I who had insulin coma on more than twenty occasions were all free from proteinuria after twenty-five years of diabetes. Four had normal ocular fundi and the remainder only a few microaneurysms. These observations suggest that frequent insulin coma does not favor retinopathy or nephropathy.

SUMMARIO IN INTERLINGUA

Retinopathia e Nephropathia in Diabete Mellite: Comparation del Effectos de Duo Formas de Tractamento

Esseva revistate ab le puncto de vista del presentia de nephropathia e retinopathia omne le casos de diabete discoperite a Malmö in patientes de minus que quaranta annos de etate durante le periodos (I) ab 1922 usque a 1935 e (II) ab 1936 usque a 1945. Serie I consisteva de septanta-un patientes, serie II de 115. Esseva excludite le casos de patientes morte post historias clinic troppo curte pro le disveloppamento de complicationes de diabete. Isto reduceva le series a cinquanta-quatro e 105 patientes, respectivemente. Dece-novem del patientes in serie I e cinque in serie II habeva morite ab complicationes tardive de diabete. Omne le patientes, con un sol exception in serie II, habeva essite examinate per le autor in persona.

Le frequentia de nephropathia in serie I esseva significativemente plus basse que in serie II, e isto in despecto del facto que le duration medie del presentia de diabete in serie I excedeva illo in serie II per octo annos e medie. Iste cifras include le patientes morte ab complicationes tardive de diabete.

Nulle significative differentia esseva constatate in le frequentia de retinopathia inter le duo series. Le differentia del duration medie del presentia de diabete inter le duo gruppos esseva dece annos e medie. Tamen, con respecto al grado de severitate del condition il esseva constatate que sever retinopathia e sever compromisso de vision esseva significativemente plus commun in serie II, ben que le duration medie del presentia de diabete in le secunde gruppo esseva dece annos minus extense que in le prime. Iste cifras include solmente

patientes qui esseva vive al tempore del revista.

Durante le prime periodo le patientes recipeva dietas strictemente restringite a basse contentos de hydrato de carbon e basse contentos caloric. Verso le fin del quarte decennio de nostre seculo, le restrictiones dietari deveniva multo minus rigide, e un pauco plus tarde le patientes esseva emancipate completemente con respecto al selection de lor dietas. A ille tempore nulle effortio esseva facite a reducer le glycosuria del patientes al plus basse nivello possibile. In serie I, le dieta del patientes esseva strictemente regulate durante un periodo medie de dece annos; illo esseva libere durante, al media, 18 pro cento del curso total del diabete. In serie II, le dieta del patientes esseva strictemente regulate durante un periodo medie de duo annos e medie; illo esseva libere durante, al media, 85 pro cento del curso total del diabete.

Durante un periodo medie correspondente a 10 pro cento del periodo total del diabete, le patientes in serie I recipeva solmente un injection diurne de insulina. Isto es a comparar con 45 pro cento in serie II. Tanto le frequentia de coma insulinic como etiam le incidentia de reactiones a insulina esseva significativemente plus alte in serie I que in serie II. Le pesos corporee in serie I esseva significativemente plus basse al tempore del revista que in serie II. Le datos relative a dieta e dosage de insulina rende multo probabile que le grado de hyperglycemia in serie I esseva plus basse que in serie II.

Le revista pare indicar que le meticulose regulation del diabete retarda le disveloppamento de nephropathia e de retinopathia.

Le octo patientes in serie I qui experientiava coma insulinic a plus que vinti occasiones esseva omnes libere de proteinuria al fin de vinti-cinque annos de diabete. Quatro habeva normal fundos ocular, e le alteres habeva solmente pauco numerose microaneurysmas. Iste constatationes suggere que le frequente occurrentia de coma insulinic non promove le disveloppamento de retinopathia o de nephropathia.

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Retinal Capillary Microaneurysms

A Concept of Pathogenesis

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In 1888, Nettleship[1] discovered microaneurysms in diabetic retinas, but not until more than half a century later were they rediscovered, in 1943.[2] The capillary microaneurysm is now considered one of the earliest pathognomonic lesions if not the initiating event of diabetic retinopathy. Pathologically the smallest microaneurysm is a saccular dilatation arising from one side of a capillary. In their 1943 report Ballantyne and Loewenstein described lipid within the walls of the capillaries and aneurysms.[2] Friedenwald, by histologic section, demonstrated splitting of the basement membrane at the site of the aneurysm.[3] Since these early papers voluminous literature has developed concerning the aneurysms and closely related subjects. The literature was extensively reviewed by Wagener[4] in 1952 and by Ashton[5] in 1958.

This study endeavors to disclose additional histologic facts about microaneurysms by employing a battery of routine stains. Presented here is a concept of the pathogenesis of microaneurysms based on a post-mortem study employing these routine stains on the eyes of four diabetic subjects.

METHODS

At autopsy the posterior half of each eye was removed through the unroofed orbit and fixed in 10 per cent formaldehyde. Following fixation by immersion for at least forty-eight hours, portions of the retinas were imbedded in paraffin as nearly flat as possible and serially sectioned, usually at 10 μ. The retinas of four diabetic subjects and four nondiabetic patients were examined and compared. Each retinal block provided 15 to 30 sections which were stained by a variety of methods. The most useful technics in this study proved to be the Schiff procedure (PAS) and the reticulin stain (Wilder's). In addition, frozen sections and "whole mounts" stained with Oil Red O were prepared. The frozen sections varied in thickness from 10 μ to as high as 25, the latter having the advantage that abnormal deposits of fat were more completely retained in situ than they were in the thin preparations.

The eyes regarded as normal were removed from nondiabetic patients who had had no evidence of glycosuria or hyperglycemia at any time during their hospital stay. Family history of diabetes was also denied. The diabetic retinas were removed from patients with long-standing diabetes who clinically had diabetic retinopathy. Some pertinent data are summarized in table 1.

RESULTS

In the walls of normal retinal capillaries, at least three layers of PAS-positive material were visualized by light microscopy. Collectively these three or more are referred to as the basement membrane. Internal to the latter,

TABLE 1

Diabetic patients

Number	Age	Sex	Duration of diabetes (years)	Other diseases	Renal disease
1	75	F	25	Chronic monocytic leukemia	Intercapillary glomerulosclerosis
2	57	M	10	Acute myocardial infarction	Intercapillary glomerulosclerosis
3	61	M	16	Cirrhosis	Intercapillary glomerulosclerosis Ureteral calculi with hydronephrosis
4	36	M	23	Acute myocardial infarction Uremia	Intercapillary glomerulosclerosis
			Nondiabetic patients		
1	52	M		Undifferentiated malignant tumor	none
2	32	M		Traumatic death	none
3	38	F		Subacute bacterial endocarditis	none
4	4	M		Acute leukemia	none

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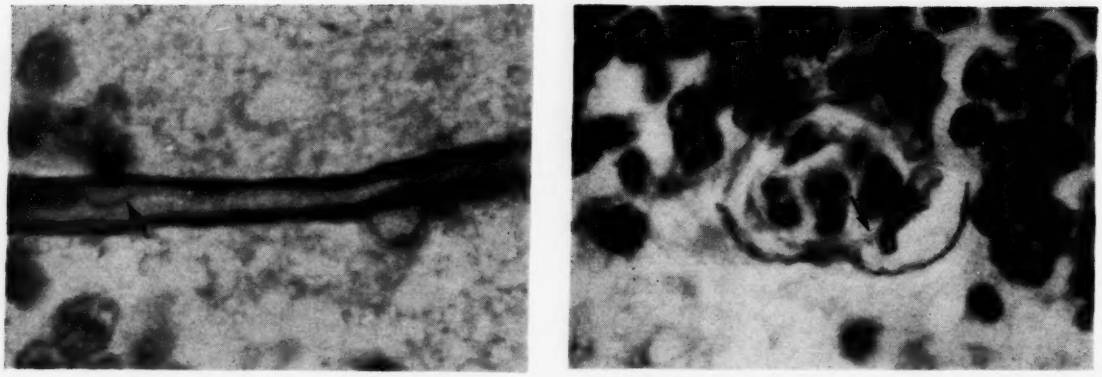


FIG. 1. Left—On the left side an endothelial nucleus is seen within the PAS-positive basement membrane. Right—On the right side of a nondiabetic capillary aneurysm, an endothelial nucleus is covered with a thin layer of PAS-positive material on the luminal side of this nucleus. PAS stain × 1,700.

flattened, elongated nuclei of endothelial cells were found and, in favorable sections, a very thin layer of PAS-positive material covered their luminal borders (figure 1). The cytoplasm of normal endothelial cells was rarely visualized in the retinas, but it may become more prominent in the presence of diabetes. Possibly the PAS-positive material itself may be part of the endothelial cells, as suggested by other tissues prepared by silver nitrate injection.[6] There are small round nuclei on the outer surface of the capillary which may produce supportive reticulum fibers. External to these cells is a layer of PAS-positive material of moderate thickness which is continuous with the basement membrane described above (figure 2). Reticulum runs approximately parallel to the capillary, with interlacing strands oriented tangentially to its long axis (figure 3). Beyond this, the capillary is surrounded by elements of nerve cells and has no visible support other than reticulum. No fat was seen within the walls of the capillaries but was occasionally noted in the arterioles of the oldest subject.

In the retinas from diabetic subjects, abnormal deposits of stained fat within the walls of capillaries and aneurysms were a prominent feature, confirming earlier reports.[2,7] Spaces between the layers of the PAS-positive basement membrane in the aneurysmal walls were abnormally widened and here stainable lipid was often present (figures 4 and 5). In advanced stages with advent of hyalinization, these spaces tended to become obliterated forming a dense PAS-positive cap. In some aneurysms erythrocytes are also seen between the layers of the basement membrane (figure 6). Retinal microaneurysms in diabetic patients are not seen to possess surrounding reticulum although in adjacent areas of the capillary it is normal (figure 7). In frozen sections from

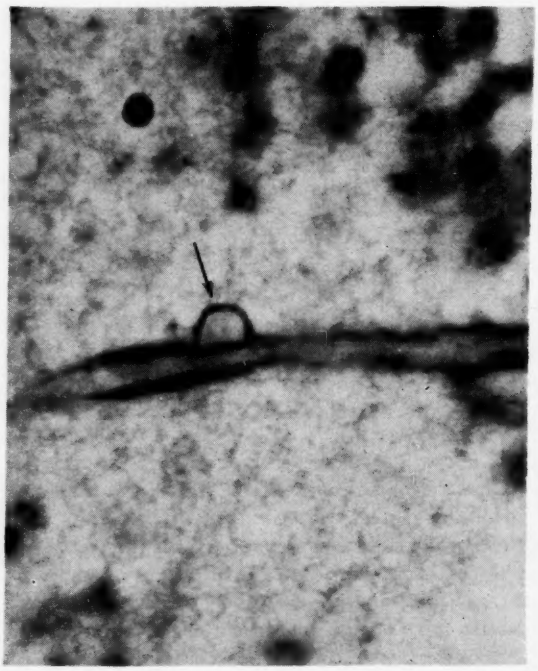


FIG. 2. A cell nucleus covered with a layer of PAS-positive material, fused on either side of this nucleus. PAS stain × 1,700.

one diabetic, stained with Oil Red O, intraluminal fat plugs of varying density ("fat emboli") were demonstrated in capillaries (figure 8). In some areas deposition of similar lipid may be seen in the endothelial cells. Rarely one sees a capillary focally distended to three or four times its normal diameter by lipid without aneurysm formation.

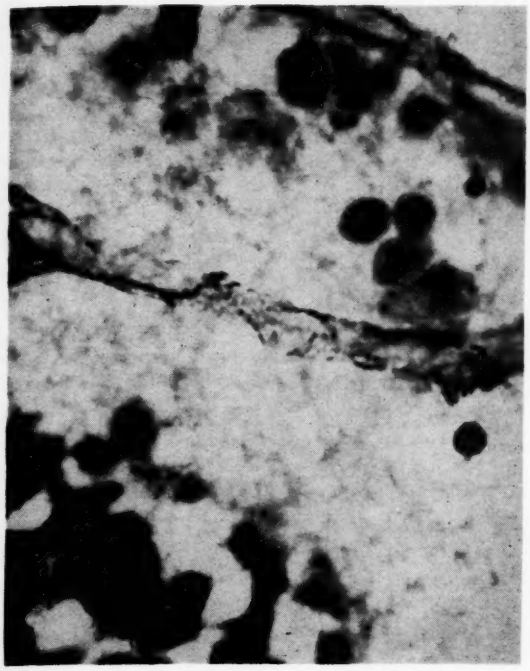


FIG. 3. Web of reticulum covering a retinal capillary. Wilder's reticulum stain × 1,700.

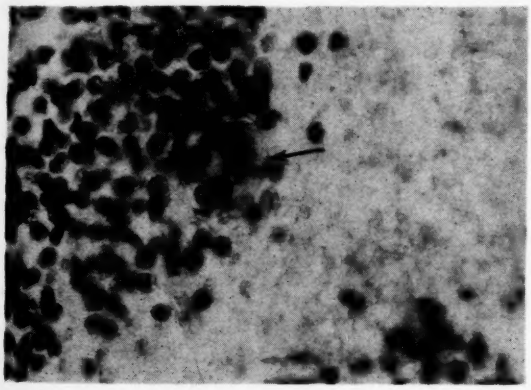


FIG. 4. Diabetic capillary microaneurysm. The dark material represents fat within the wall of the aneurysm. Oil Red O stain × 940.

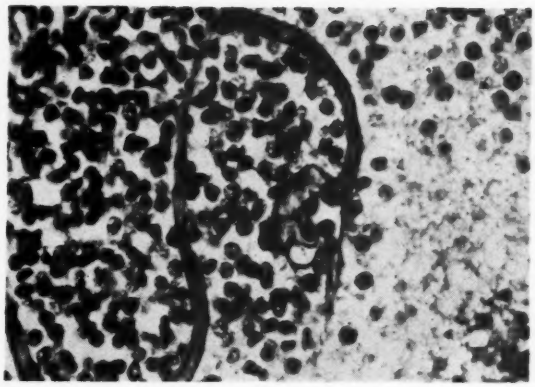


FIG. 5. Diabetic small aneurysm with splitting of the basement membrane. PAS stain × 940.

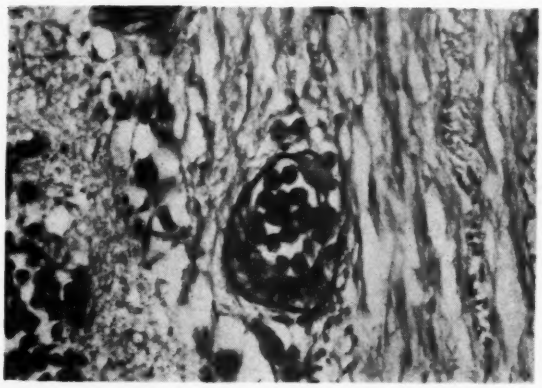


FIG. 6. Erythrocytes within the wall of a diabetic aneurysm. PAS stain × 940.

DISCUSSION

Ashton[8,9] has suggested that microaneurysms in the diabetic develop by looping with fusion and absorption of the opposed capillary walls. Although this sequence might occur, reticulum might be expected to be carried with the wall and would then cover the cap of the aneurysm. But in the retinas reported here, all of the aneurysms studied lacked reticulum.

If these lesions are "true" aneurysms (i.e., an actual bulge of the capillary wall) and not varicose loops in which opposing walls are absorbed, the lining must have been weakened, or pressure from within must have been increased, or both. Ballantyne[7] suggests that fat in the endothelial cell is an early change indicating damage to the endothelium so that the cells are less able to withstand pressure than normal cells.

It is assumed that reticulum provides some support for the capillary wall. It is absent in walls of aneurysms; the latter are, therefore, at least in one sense herniations of the other elements of the capillary wall through adventitial reticulum. Additional support for this hypothesis is offered by the uniform acuteness of the angles formed by the wall of the aneurysms with that of the neck. The openings of the aneurysms into the capillaries are surrounded by reticulum fibers arranged

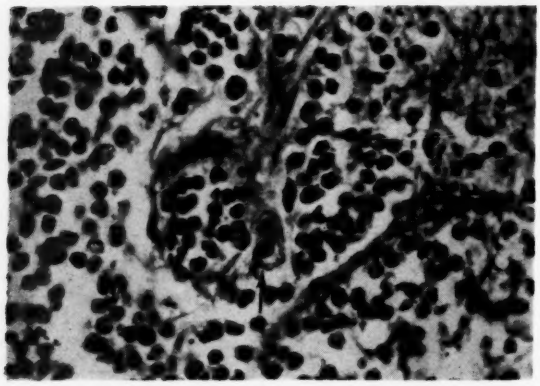


FIG. 7. Diabetic aneurysm without a reticulum covering; the capillary from which it arose is surrounded by normal reticulum. Wilder's reticulum stain × 940.

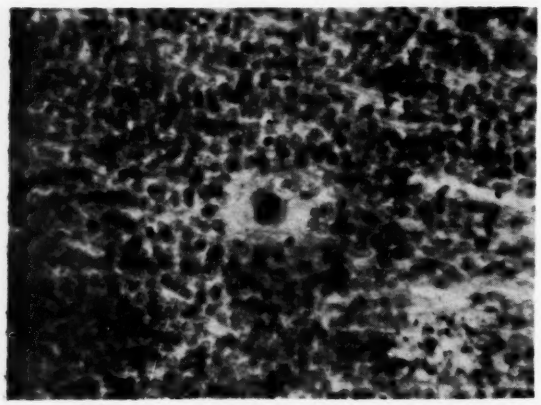


FIG. 8. Lipid within a capillary of a diabetic retina (homogenous gray). Oil Red O stain of frozen section × 430.

so as to suggest that a portion of the wall had bulged through a gap in the reticulum network.

Some mechanism must operate to produce sufficient widening of the network for herniation through it. Possibly when abnormal deposits of mural and intra-endothelial fat in capillaries of the diabetic become sufficiently large they expand the vessel at this site so greatly that the wall herniates through the open meshwork of the reticulum.

Lipid within the capillary wall may well come from its lumen although other sources can be postulated. If from the blood it is from either serum lipids present in increased amounts or from pathologic fat droplets (small emboli or perhaps abnormally large chylomicrons). Total serum lipids are increased in patients with retinopathy[10] and the lesions are seen on the venous side of the capillary, suggesting that the fat may have been accumulated from serum lipids under an increased venous pressure as suggested by the distention of the veins in diabetes noted by Nettleship[3] and subsequently by others. Some factors, however, favor the possibility of actual "fat embolism." The focal fat absorption seen in the capillaries would suggest a localized collection of endovascular lipid. "Fat emboli" have been described in kidneys of diabetic man[11] and in the lung.[12] They also occur within retinal capillaries.

Once a small aneurysm is formed, progressive enlargement may be expected. The hyaline cap noted by many authors may be similar to the nodules of intercapillary glomerulosclerosis where it has been suggested that the nodules are originally fat which has been slowly replaced by hyaline.[13] Conversion of mixtures of blood and fat to a PAS-positive hyaline material does occur in choline-deficient rats.[11] Friedenwald[5] has suggested that retinal microaneurysms and Kimmelstiel-Wilson nodules might have a similar pathogenesis. The finding of a few aneurysms in diabetic glomeruli emphasizes their probable similar pathogenesis.[14]

Reasons for localization of these lesions to the retina can only be suggested. Perhaps several local factors operate together to produce the necessary absorption of fat. Venous stasis or engorgement is a well-known feature of diabetic retinopathy.[4] The endovascular pressure within the retina must be somewhat higher than elsewhere in the body in order to be greater than intraocular tension.[15,16] Superimposition of this stasis and increased pressure on the hyperlipemic state may well cause absorption by endothelial cells, either directly from the serum or a fat embolus. Perhaps, in addition, capillaries of the retina must depend almost solely on their own inherent properties for support in contrast to other tissues, and once this support is lost, an aneurysm could develop at this site.

This hypothesis is schematically represented in figure 9. In a stepwise manner, "A" represents a normal capillary. "B" depicts fat in the capillary lumen, either as inspissated serum lipids or as a "fat embolus." "C" represents fat absorbed within the wall which is probably responsible for some, if not all, of the splitting of the basement membrane. Distention of the capillary wall by absorbed fat is seen in "D." Here the bulk of fat could overdistend and thin out the reticulum. "E" is the final stage similar to figure 3 with its lack of reticulum.

SUMMARY

A concept of the pathogenesis of some capillary microaneurysms of the retinas in diabetes has been presented. Evidence is presented to support the hypothesis that the microaneurysms are instituted by accumulation

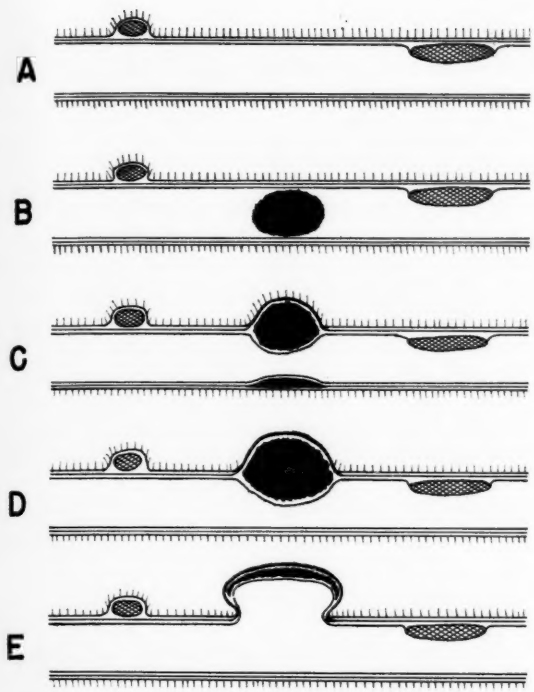


FIG. 9. A. A normal capillary is composed of PAS-positive material surrounded by reticulum. An endothelial cell nucleus is within the basement membrane on the lumen side and another nucleus is on the extraluminal side. Surrounding reticulum is represented by the short perpendicular lines.
B. Fat within the capillary lumen either in the form of serum lipids or as an "embolus."
C. Fat absorbed into the capillary wall (see figure 4), with splitting of the basement membrane.
D. A hypothetical stage is represented in which a large amount of fat is contained within the capillary wall producing distention beyond the reticulum supply.
E. A small full-blown aneurysm (see figure 5) with its split layer of PAS-positive material and lack of reticulum as in figure 7.

of fat into the capillary wall, with subsequent stretching so that it herniates through the supporting reticulum network. A minute aneurysm then develops by this herniation between reticulum fibers.

SUMMARIO IN INTERLINGUA

Un Conception Possibile del Pathogenese de Microaneurysmas de Capillares Retinal

Es presentate un conception possibile del pathogenese de microaneurysmas capillar occurrente in le retinas de patientes de diabete. Es citate observationes in supporto del hypothese que le microaneurysmas es instituite per le accumulation de grassia in le parietes capillar, con distension subsequente de maniera que hernias resulta a transverso le supportante tissu reticular. Alora un minuscule aneurysma se forma per iste herniation inter fibras reticular.

ACKNOWLEDGMENT

The author is deeply indebted to Dr. W. S. Hartroft for his advice during this study and for his editing of the manuscript.

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Diabetic Diarrhea with Malabsorption Syndrome

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A form of diarrhea peculiar to and occurring in the course of diabetes is well recognized. The history of its development suggests a progressive disease process passing from one stage to the next. In 1936, Bargen, Bollman and Kepler[1] first described and documented an intractable form of diarrhea of undetermined etiology in some of their diabetic patients. To differentiate this from pancreatic diarrhea they demonstrated the presence of normal exocrine pancreatic secretion. They also showed that the administration of active pancreatic juice to their patients was completely lacking in therapeutic efficacy in contrast to its successful use in cases of diarrhea resulting from chronic pancreatitis. In 1945 Rundles[2] noted the frequent association of this condition with evidences of autonomic nervous system involvement. Sheridan and Bailey,[3] 1946, added to the original description the observation that the diarrhea is intermittent, that it commonly has a nocturnal accentuation, and is often accompanied by fecal incontinence. They also indicated the frequent presence of neuropathy, and in cases with neuropathy the almost constant presence of elevated spinal fluid protein.

The next development in our knowledge of this syndrome was the demonstration by Hodges, Rundles and Hanelin[4] in 1947 of the presence of a disordered motor function ("deficiency pattern") of the small bowel on X-ray examination. There was segmentation, considerable variation of the caliber of the intestinal lumen, localized coarsening, irregularity and partial obliteration of folds with preservation of the mucosal pattern; they noted the occasional association of some degree of gastric retention. However, there was little correlation between clinical symptoms and the type or degree of roentgen abnormality.

Until the paper by Berge and his co-workers in 1956[5] there had been no reports indicating the presence of steatorrhea in diabetic diarrhea. These authors reported eight cases with steatorrhea. They emphasized that the symptoms in their cases, namely intractable diarrhea with postprandial and nocturnal exacerbation and fecal incontinence, were in no way different from those cases previously reported in which steatorrhea had not been demonstrated. They also documented the frequent postprandial exacerbation of the diarrhea. Although there was laboratory evidence of steatorrhea, the patients did not have bulky, malodorous stools and did not show clinical evidences of malabsorption syndrome. Since then other authors have reported steatorrhea in diabetic diarrhea, but again none of these cases was associated with evidences of malabsorption syndrome.[6,7]

Mailman,[8] 1958, reported a diabetic patient with diarrhea consisting of copious, malodorous stools, protuberant abdomen, marked steatorrhea up to 46 gm. per day, a disordered motor pattern of the small bowel and a remission on corticosteroid therapy. No quantitative studies were made to evaluate malabsorption although the clinical picture was suggestive.

We are presenting two patients with long-standing diabetes who had severe diarrhea with steatorrhea, plus all the accepted criteria for the diagnosis of malabsorption syndrome, and who responded dramatically to corticosteroid administration. The combination of diabetes and frank malabsorption constitutes a syndrome that has not heretofore been appreciated in the literature.

CASE REPORTS

Case 1: A forty-one-year-old white female was first admitted to the Mount Sinai Hospital in 1955 with a history of diarrhea for six months accompanied by a twenty-pound weight loss. There were three to ten bowel movements per day. Stools were described as watery and foul without blood, pus or mucus, and accompanied by mild abdominal cramps. Edema of the ankles and sensation of cramps in both hands had developed more recently. A multitude of nonspecific treatments for the diarrhea had been of no avail. There had been transitory episodes of diarrhea at age eighteen, twenty-four and thirty-four, each lasting approximately two weeks.

The past history is significant in that she was a known diabetic for thirty years with a classical onset at age eleven. Since then the diabetes had been well regulated on diet and insulin. At the time of her admission, she was well controlled on 20 units of Protamine Zinc Insulin daily. She had had two uneventful pregnancies eleven and four years before. The family history was negative for diabetes.

Physical examination revealed a chronically ill, wasted female in no distress. Weight was 90 lb. in spite of three-plus pedal edema. Blood pressure 105/60 mm. Hg. The abdomen

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was soft, doughy and slightly distended with hyperactive bowel sounds. The fundi were normal; neurologic examination was negative; peripheral pulses were readily palpable. There were no other significant findings.

Laboratory examination showed hypochromic anemia with hemoglobin of 10.8 gm./100 ml. and normal erythrocyte and leucocyte counts; platelets numbered 236,000 per cu. mm.; hematocrit was 38 per cent. Urine contained 0 to 1 plus albumin. Blood chemical studies revealed: urea 14 mg./100 ml.; total protein 6 gm./100 ml.; albumin 2.8 gm./100 ml.; globulin 3.2 gm./100 ml.; calcium 6.4 mg./100 ml.; phosphorus 3.2 mg./100 ml.; cholesterol 115 mg./100 ml.; alkaline phosphatase 9.5 King-Armstrong units; prothrombin time 27 seconds. The vitamin A tolerance test showed a marked depression, the values being: fasting, 7 μg., four hours 40 μg., six hours 10 μg., and eight hours 6 μg. The serum carotene was 16 gamma/100 ml. Stool examination revealed abnormally large quantities of fat with few fatty acid crystals; no ova or parasites were present. Pancreatic secretion studies, gastric analysis and adrenal function studies were normal. Gastrointestinal series showed changes in the small intestine consisting of coarsening of the folds, evidence of retained secretions, delayed segmentation and areas of marked dilatation (figure 1).

The patient was treated with large doses of vitamin B_{12}, folic acid and liver extract, but the diarrhea persisted. Following the use of hydrocortisone, 60 mg. daily, there was a prompt and complete disappearance of diarrhea as well as all other symptoms and findings present on admission. A rapid weight gain ensued. Although the insulin requirement rose to 60 units of Lente insulin daily, diabetes remained well controlled. The patient has been asymptomatic since her discharge four years ago and has been gainfully employed in addition to caring for her invalid husband.



FIG. 1. X ray of small bowel, Case I. Note delayed segmentation, areas of dilatation, retained secretions and coarsening of the folds.

Case II: This forty-five-year-old white female was admitted to the Mount Sinai Hospital in August 1958 because of severe weakness and pain associated with recurrent bouts of diarrhea for the past five years. The first episode of diarrhea failed to respond to various therapeutic measures but ceased coincidentally with injections of vitamin B_{12}. Several subsequent bouts of diarrhea cleared spontaneously. However, for the two years prior to this admission the diarrhea had been unresponsive to therapy, including vitamin B_{12}. The bowel movements were described as being light in color, foul-smelling, copious and as floating on the surface of the bowl water. Bowel movements varied from five to twenty per day, frequently more numerous at night and interfering with sleep. She had lost ten pounds and had developed increasingly severe and generalized bone pains. The pains had become so severe that frequent administration of narcotics was found necessary. There was progressive difficulty in ambulation so that crutches had to be employed.

Past history included the fact that she had had diabetes for thirty-five years with onset at the age of ten. During the thirty-five-year period, there had been numerous admissions to the hospital for diabetic ketosis in her early years, infections, transient thrombocytopenic purpura, therapeutic abortion and three separate episodes of oculomotor palsy. Except for the episodes of ketosis in the early years of her diabetes, this patient had been under satisfactory control.

Physical examination revealed a frail, irritable female with evident weight loss, complaining of severe pain. She was barely able to walk with the aid of crutches; weight eighty-nine pounds; blood pressure 120/70 mm. Hg., abdomen soft, protuberant with peristaltic waves visible. There was no evidence of diabetic retinopathy or vascular disease; the deep tendon reflexes were absent; there were bilateral lenticular opacities; the tongue was red and smooth. There were generalized bone pain and tenderness.

Laboratory studies revealed hemoglobin 9.6 gm./100 ml.; leucocyte count was normal; urine contained 0 to 1 plus albumin. Blood chemistries: urea 9 mg./100 ml.; total protein 6.7 gm./100 ml., albumin 3.5, globulin 3.2; calcium 9.4 mg./100 ml.; phosphorus 3.5 mg./100 ml.; alkaline phosphatase 31.6 King-Armstrong units; cephalin flocculation 2 plus; thymol turbidity 0.8 units. Vitamin A tolerance test showed a marked absorption defect, the values being: fasting, 5 μg.; four hours, 20 μg.; six hours, 8 μg.; and eight hours, 6 μg. Blood carotene level was 4 gamma/100 ml.; prothrombin time 40 seconds, control 12 seconds. Stool fat was abnormally increased to 24 gm. per day. Gastric acidity and pancreatic function studies were normal. Gastrointestinal X rays showed generalized dilatation of the jejunum and ileum with evidence of coarse flocculation in the jejunal loops (figure 2); X rays of the bones showed demineralization, multiple fractures and Milkman's zones (figures 3, 4). Biopsy of the jejunal mucosa showed the presence of clubbing and flattening of the villi (figure 5) as described in malabsorption syndrome. Lumbar puncture revealed elevation of the spinal fluid protein to 73 mg. per cent.



FIG. 2. X ray of small bowel, Case II. Note dilatation of jejunem and ileum with evidence of coarse flocculation and retained secretions.

Corticosteroid therapy was instituted with methylprednisolone, 16 mg./day. There was a prompt and dramatic response. The diarrhea ceased, the patient's appetite returned, her weight and strength increased and her pain gradually diminished. Within one month she no longer required narcotics for her pain and shortly thereafter was able to discard her crutches. At the present time, almost one year later, she is asymptomatic on 4 mg. methylprednisolone daily. During all this time the diabetes has remained under satisfactory control with an increase in the daily requirement of Isophane insulin from 40 to 55 units.

DISCUSSION

These cases clearly demonstrate marked malabsorption occurring in two diabetic patients with diarrhea (table 1). The problem of the pathogenesis of the syndrome suggests several possibilities. It may be the result of: (1) a coincidental association of diabetes and the sprue syndrome or (2) a manifestation of diabetes per se resulting from neuropathic, vascular or metabolic involvement.

The possibility of a coincidental association of these two diseases to explain the clinical picture in our two cases cannot be denied. However, in addition to the fact that this combination has not been previously documented, there are some features that suggest the possibility of a different conclusion. For example, it must be pointed out that sprue is not a disease but is a syndrome. As such, there has been an ever-growing number of specific diseases that have been established within the framework of the sprue symptomatology. This group of established etiologies is now classified as "secondary" sprue whereas the remainder are called "primary" sprue. The category of secondary sprue includes total or partial gastrectomy,[9] lymphoma,[10] Whipple's disease,[11] pancreatic insufficiency,[10] duodenal and jejunal diverticulosis,[10] amyloidosis,[10] regional enteritis,[12] infarction of the bowel,[13] gastrointestinal anastamoses,[14] agammaglobulinemia,[15] and ulcerogenic tumor of the pancreas.[16] It would, therefore, not be a novel departure if our cases were considered to have a specific etiology. The biopsy of small intestinal mucosa with the characteristic blunting and clubbing of the villi is not necessarily specific for sprue but has been demonstrated postgastrectomy[17] and in other nonsprue conditions.[18] Strongly suggestive of a possible diabetic association is the striking fact that both our cases, as well as that of Mailman, all had early onset with markedly prolonged duration of diabetes, each over thirty years. Nevertheless they demonstrated remarkably few evidences of the vascular degenerative changes associated with diabetes of long duration (table 2). This suggests that the syndrome as herein described could represent a peculiar

TABLE 1

Diagnostic laboratory findings

	Case I	Case II
Steatorrhea	+	+
Secretin test	N	N
Gastric acidity	N	N
Carotene level	16γ%	4γ%
Vitamin A tolerance curve	Flat	Flat
Prothrombin time	27 sec.	40 sec.
Calcium	6.4	9.1
Phosphorus	3.2	3.0
Total protein	6.0	6.7
Alkaline phosphatase (KA units)	9.5	31.2
Cholesterol	115	204
Small bowel series	+	+
Osteomalacia	—	+
Jejunal biopsy	—	+

TABLE 2

Duration of diabetes and evaluation of degenerative changes

	Case I	Case II	Mailman's Case
Age of onset	11	11	22
Duration of diabetes	30	35	35
Diabetic retinopathy	0	0	0
Blood pressure	100/60	130/80	120/60
Albuminuria	0 - 1+	0 - tr.	tr.
Blood urea	10 mg. %	9 mg.%	—
Neuropathy	0	+	+



FIG. 3. X ray of pelvis, Case II, showing fractures as well as demineralization of the bone.

stage of progression of the diarrhea which presumably occurs only in patients who survive long enough and with very few other involvements to interfere with its development. This concept is consistent with the historical development as it unfolded with the prolongation of life following the institution of insulin therapy.

Assuming that this picture is a manifestation of diabetes, the question of mechanism arises. Berge and his colleagues,[19] in a carefully controlled postmortem study of the intestinal tract, were unable to demonstrate any mucosal, muscular, neurologic or vascular involvement. Nevertheless, most authors who have studied this complication believe that the diarrhea in diabetes is a manifestation of autonomic nervous system involvement. This is based on the frequent occurrence of associated involvement of other components of the autonomic system as evidenced by neurogenic bladder, orthostatic hypotension and demonstrable changes in the sweating pattern.[20,21] Further, the X-ray changes are compatible with those seen after some instances of vagotomy[22] and not infrequently following resection of the sympathetic ganglia.[23] Against this attractive theory is the occasional absence of any demonstrable evidence of any neuropathic involvement, such as occurred in two out of eight cases in one report[5] and in our first case.

That this could be vascular in origin is also a possibility, particularly in view of the frequency of degenerative changes of the blood vessels in diabetes. However, in addition to the lack of demonstration of this finding at autopsy,[19] it is significant that our patients were relatively free from any stigma of major vascular involvement. Whether or not this is a "metabolic" manifestation is completely hypothetical.

Regardless of the pathogenesis of the clinical picture, the therapeutic achievement in these cases is most gratifying. Our patients have been restored to a normal,



FIG. 4. X ray of tibia and fibula, Case II. Note presence of Milkman's zones and demineralization.

useful life situation instead of severe diarrhea, sleepless nights, bone pains requiring narcotics for relief, and invalidism. Corticosteroid administration produced a reversal of the intestinal symptoms and the associated clinical and laboratory evidences of malabsorption. This contrasts with the inefficacy of this type of therapy in other forms of diabetic diarrhea.

The importance of recognizing this type of diarrhea in the diabetic, namely steatorrhea with malabsorption syndrome, in the light of the attainable therapeutic result hardly requires further elaboration. This is even more demanding when one considers the relative lack of any dependable, consistent therapeutic approach to other forms of diabetic diarrhea.

SUMMARY

1. Two diabetic patients who developed diarrhea with steatorrhea and malabsorption syndrome are presented.

2. Both patients responded dramatically to corticosteroid administration.

3. The pathogenesis of this syndrome is discussed. It is suggested that it may be conditioned by diabetes of prolonged duration in the absence of significant degenerative changes.

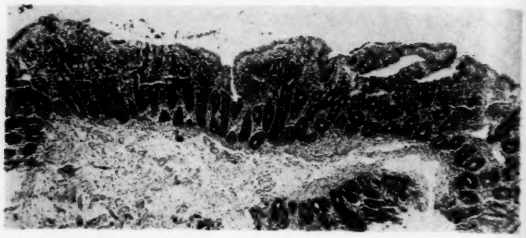


FIG. 5. Biopsy specimen of jejunem in Case II. Note clubbing and flattening of villi.

SUMMARIO IN INTERLINGUA

Diarrhea Diabetic con Syndrome de Malabsorption

1. Es presentate le casos de duo patientes de diabete qui disveloppava diarrhea con steatorrhea e syndrome de malabsorption.

2. Ambe patientes respondeva frappantemente al administration de corticosteroide.

3. Le pathogenese de iste syndrome es discutite. Es opinate que illo pote esser conditionate per diabete de duration prolongate in le absentia de significative alterationes degeneratori.

ACKNOWLEDGMENT

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Diet and Glucose Tolerance

The work of Chernick and Scow indicates that factors other than the diet play an important role in regulating the rate of removal of glucose from the blood. They found that hypophysectomy markedly reduced this rate, especially during the first thirty minutes after injecting the glucose. The slopes of the curves (log of excess blood glucose versus time), and therefore the rates for glucose removal, are the same for both the operated and control rats during the second half of the glucose tolerance period (thirty to seventy-five minutes). This observation suggests that during the course of the glucose tolerance, some physiological change(s) may have occurred in the processes controlling the blood sugar level.

The above investigators point out that the blood glucose level at any particular moment is the result of two opposing forces, those adding glucose to, and those removing glucose from, the blood. The first of these factors is probably related to the level of glycogen in the liver, while the latter is probably influenced by the peripheral uptake of glucose. A consideration of these factors is important before one can accept the suggestion that a new dietary component exists which regulates the removal of glucose from the blood. No evidence has been presented by Schwarz and Mertz as to the influence of their diets on the liver glycogen stores of the rats. There is a possibility that something inherent either in their experimental procedure or diets may be responsible for some of their observed effects.

The work of Uram, Friedman and Kline may possibly be explained by variations in the rate of absorption of the glucose from the intestinal tract of the animals fed the different diets. They suggest that the oatmeal diet used by von Noorden fifty years ago for treating diabetic individuals was supporting evidence for their hypothesis that a dietary factor exists which regulates the removal of glucose from the blood. C. von Noorden (*Clinical Treatise on the Pathology and Therapy of Disorders of Metabolism and Nutrition*, E. B. Treat & Co., New York, Part VII, 1905) showed that a diet containing 250 gm. of oatmeal per day reduced the urinary glucose excretion in severe cases of diabetes. Recent reports suggest that the improved condition of the diabetic individuals fed the oatmeal diet (or the rice diet of von Duering, or the potato diet of Mossé; see von Noorden, *loc. cit.*) is due to the inanition that frequently accompanied the dietary regimen (G. G. Duncan in *Diseases of Metabolism*, edited by G. G. Duncan, W. B. Saunders Co., Philadelphia, 1952).

From an historical standpoint, it may be worthy of note that one of the early reports on glucose tolerance curves appeared in the paper by A. T. B. Jacobsen of Copenhagen (*Biochem. Ztschr.* 56:471, 1913). He found that the blood sugar levels of his normal men showed what we now recognize as a normal glucose tolerance curve only when carbohydrate foods (glucose and starch) were fed; fats (olive oil) and protein (gelatine) produced no change in the blood glucose level. Three to four hours after breakfast, he took a blood sample and then gave the subject from 80 to 100 gm. of the test foods. Additional blood samples were taken at one-half hour intervals thereafter. When the blood sugar values were plotted against time, the curves for the carbohydrate meal were similar to those that we now associate with a typical glucose tolerance.

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Blood Groups and Diabetes Mellitus

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Aird, Bentall and Roberts' discovery[1] of a relationship between the incidence of gastric carcinoma and blood group A promoted a number of studies on the relationship between blood groups and various diseases, such as duodenal ulcer, gastric ulcer, gastric carcinoma, pernicious anemia, cancer of the colon and rectum, cancer of the breast, cancer of the lung, and diabetes.[8] In the three first-mentioned diseases the evidence supplied by the different investigators appears to be so strong and consistent that it seems fairly certain that a relationship exists between duodenal and gastric ulcer and Group O as well as between gastric carcinoma and Group A. There are marked indications also that Group A persons are particularly apt to develop pernicious anemia.

There is fairly strong evidence that diabetes mellitus is commoner in people of Group A, but as to the other diseases mentioned the results are negative.

In 1955 and 1956 two papers on the distribution of blood groups among diabetics were published. Craig and Wang[4] performed ABO blood groupings on 817 diabetic patients treated at the Victoria Infirmary, Glasgow. The series as a whole, as well as classified by sex, age, and weight when diabetes was diagnosed, showed no significant differences from a control series of blood donors. By dividing the series into various subgroups, the authors succeeded in demonstrating that all overweight patients, as well as patients with a family history of diabetes or obesity, showed a significantly reduced frequency of Group O as compared with all other blood groups combined. This was particularly marked when both factors were combined. Conversely, all nonobese diabetics and all male diabetics without a family history of diabetes showed a significant predominance of Group O compared with all other blood groups. The authors conclude that without confirmation from other series the divergences which they found cannot be interpreted as more than possible factors in "the natural history of diabetes mellitus."

In an analysis of 1,333 patients with diabetes mellitus from Liverpool and Oxford, McConnell, Pyke, and Roberts[3] found that among male diabetics there was a marked increase in the relative number of Group A persons compared with the control series which consisted of donors from the respective areas. Diabetic women, on the other hand, did not show significant divergences from the control series. Among the males the excess of Group A was greatest among the young patients whose diabetes began before the age of thirty, but the difference between this group and the other groups was not significant. On the other hand, both groups, combined as well as separately, differed significantly from the control series. The excess of Group A among the males was far more marked in cases where a close relative also suffered from diabetes. On the basis of these findings, the authors advance the hypothesis that the excess of Group A among the males may be characteristic of a special type of diabetes rather than of the disease as a whole. They do not consider the difference in the distribution as definitely proved, and only submit it for further trial.

The Oxford series was also investigated from the point of view of the distribution of Rh positive and Rh negative as well as MN types which did not differ significantly from the control series.

Since the results of these two British investigations are to some extent conflicting, it was decided to study a Danish series of diabetics to find out whether the ABO groups among Danish diabetics differ from those among the general Danish population. In addition, it was decided to investigate the frequency of the Lewis group Le(a+) in the Danish diabetic series to ascertain whether a relationship exists between diabetes and the secretor status Le(a+) persons are nonsecretors of blood group substances A, B, and H. Lastly, it was considered expedient to combine the ABO grouping with a routine Rh grouping.

PRESENT INVESTIGATION

Diabetic Series. The series comprises 992 diabetics treated at the Hvidøre Hospital, Copenhagen, during the period Aug. 20, 1956, to Sept. 20, 1958. The diagnosis was based on considerably elevated fasting blood sugar or a glucose tolerance curve of a diabetic type. A relatively large proportion of the patients, viz. 46 per cent, were under thirty years of age at the onset of diabetes. Classification by residence was evenly distributed over the whole of Denmark.

From The Blood Group Department, Statens Seruminstitut, Copenhagen, and the Hvidøre Hospital, Copenhagen.

Control series. As control material we used primarily a medicolegal series of 12,122 blood samples.[5] In this series, which is derived from all parts of Denmark, but with some preponderance for the cities, particularly Copenhagen, the distribution of the blood groups is given separately for males and females. Moreover, we used another five series comprising 27,332 donors from all Denmark, but with some preponderance for Copenhagen, and 9,682 patients, mainly from Copenhagen. In these five series the blood groups are not stated separately for males and females. The six series do not differ significantly from each other so far as the distribution of the ABO groups is concerned (table 1).

TABLE 1
Control series

Origin of material and author	No.	O	A	B	AB
All Denmark, paternity cases (males), Gürtler[5]	7,122	41.4	44.6	10.4	3.6
All Denmark, paternity cases (females), Gürtler[6]	5,000	41.9	43.5	10.1	4.5
Copenhagen, 2,784 donors, 6,754 patients, Jordal[6]	9,538	42.0	43.5	9.8	4.7
Copenhagen, donors, Bryde-Andersen[2]	14,304	40.6	44.0	10.9	4.5
Fifteen provincial towns, donors, Sørensen[9]	9,200	41.4	41.2	11.3	6.1
Sønderborg County Hospital, donors, Sørensen[9]	1,044	40.4	41.7	12.1	5.8
Sønderborg County Hospital, patients, Sørensen[9]	2,928	43.1	43.2	10.0	3.7
Total material	49,136	41.4	43.3	10.6	4.7

The distribution of the ABO groups in the total control series must be considered representative of the ABO distribution in Denmark.

As a control on the distribution of the Rh groups, Rh positive (D positive) and Rh negative (D negative) in the diabetic series we used Lyndrup's series of 4,319 Rh groupings[7] which consisted of 2,701 mothers and 1,618 men, mainly from paternity cases. This series is derived from all parts of Denmark. The mothers and the men, each category considered separately, must be regarded as random samples of the population as far as the distribution of blood groups is concerned. Lyndrup found 81.4 per cent Rh positive and 18.6 per cent Rh negative.

As a control on the distribution of the Lewis groups Le(a+) and Le(a—) in the diabetic material we used Jordal's series from 1957[6] consisting of 5,208 blood samples from adult men and women studied in connection with paternity cases. These samples were derived from the whole of Denmark and, according to Jordal, they did not differ significantly in regard to ABO distribution from values reported by Gürtler[5] and Andersen.[2]

TECHNIC AND RESULTS

All the blood groupings were carried out in the Blood Group Department of Statens Seruminstitut on blood samples drawn by venipuncture. Two drops of test serum were mixed with one drop of a 3-4 per cent suspension of red cells in saline in a round-bottomed tube (10 x 70 mm.). After two hours' incubation at 20°C. (ABO and Lewis grouping) or at 37°C. (Rhesus grouping) the readings were taken with the naked eye while cautiously shaking the tubes. All the test sera used contained "complete" or saline-agglutinating antibodies. The results are given in table 2.

ABO System. Table 3 shows a preponderance of Group O in male as well as female diabetics compared with the control series. Although there is no difference between the ABO groups of males and females, the males showed a significantly larger number of Group O persons than did the controls ($P < 1$ per cent).

In table 4 the diabetic series is classified into groups, age at the onset of diabetes and the frequency of Group O compared with the frequency of the other three groups combined in each age group.

Table 4 shows that there is a tendency for the excess of Group O to manifest itself especially in cases with early onset of diabetes. If the series is divided according to whether the disease set in before or after the thirtieth year of age, as did Craig and Wang[4] and McConnell, Pyke, and Roberts,[3] there is a significant excess of Group O in males as well as females with an onset of diabetes prior to the age of thirty. That the ABO distribution in the total female series does not differ significantly from the controls may be due to the fact that a large proportion of the women did not develop diabetes until after the thirtieth year of age. The men as a whole showed a significant excess of persons with Group O. In those whose diabetes had not set in until after the thirtieth year of age, however, this excess of Group O persons was not significant compared with the control series.

There is no correlation between a family history of diabetes and the distribution of ABO blood groups, either for the entire series, or for the separate age groups. A family history of diabetes was elicited in about half the cases, evenly distributed over all four ABO groups (table 5).

Further subdivision of the series according to the nature of treatment or the presence of complications was not believed to serve any purpose.

TABLE 2

Distribution of ABO, Rh, and Le(a+) groups in 992 diabetics

	O		A		B		AB		Rh-positive		Rh-negative		Le(a+)		Le(a—)	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Males	246	48.34	203	39.88	44	8.64	16	3.14	425	83.50	84	16.50	114	22.40	395	77.60
Females	217	44.93	198	40.99	50	10.35	18	3.73	398	82.41	85	17.59	117	24.22	366	75.78
Both sexes	463	46.67	401	40.42	94	9.48	34	3.43	823	82.96	169	17.04	231	23.29	761	76.61

TABLE 3

ABO distribution in the diabetic series compared with the control series

Males	Observed distribution	Computed distribution I*	Computed distribution II†	χ^2 test I*	χ^2 test II†
O	246	210.73	210.73	5.903	5.903
A	203	220.40	227.01	1.374	2.539
B	44	53.95	52.94	1.835	0.294
AB	16	23.92	18.32	2.632	0.294
Total	509	509.00	509.00	11.744	9.030

Females	Observed distribution	Computed distribution I	Computed distribution II	χ^2 test I	χ^2 test II
O	217	199.96	202.38	1.452	1.056
A	198	209.14	210.11	0.593	0.698
B	50	51.20	48.78	0.028	0.031
AB	18	22.70	21.74	0.973	0.643
Total	483	483.00	483.01	3.046	2.428

*Computed on basis of combined control series using the following ABO distribution: O: 41.4; A: 43.3; B: 10.6; AB: 4.7.

†Computed on the basis of Gürtler's series, with the following separate distribution of ABO groups: *Males*: O: 41.4; A: 44.6; B: 10.4; AB: 3.6. *Females*: O: 41.9; A: 43.5; B: 10.1; AB: 4.5.

Rhesus System. As evident from table 2, eighty-four out of 509 male diabetics (16.50 per cent) were Rh negative, whereas the corresponding value for the females was eighty-five out of 483 or 17.59 per cent. In the control series of 4,319 males and females (not specified) 18.6 per cent were Rh negative. The deviation from the control series was 1.16 times the mean error for the males and 0.54 times the mean error for the females. In other words, the distribution of Rh positive and Rh negative persons in the diabetic series does not differ significantly from the control series.

Lewis System. Out of the 509 male diabetics 114 or 22.40 per cent were Le(a+) and the females 117 of 483 or 24.22 per cent. Jordal's findings[6] were as follows: 530 out of 3,070 males or 17.26 per cent and 392 out of 2,138 females or 18.33 per cent. Comparison between the diabetic and the control series gave the result that for the males the deviation was 2.79 times the mean error and for the females 2.96 times the mean error.

For the males as well as the females there was an increased frequency of Le(a+) group as compared with the control series. These differences were highly significant ($P = .0003$).

The Lewis group determinations were not carried out by the same technic in the diabetic series and in the control series. Jordal[6] used a slide technic, one drop of test serum and one drop of a 5 per cent suspension of red cells mixed on a glass slide and left in a moist chamber for twenty minutes. Jordal states that only definitely positive reactions were designated as positive. Since frequently divergences in the incidence of the Le(a+) group are explicable by weak reactions, presumably due to "cross reaction" with "strong" Le(a—b+) cells, the diabetic series was analyzed to ascertain whether the 231 Le(a+) reactions included some which might be characterized as weak. We found twenty which were weaker than the other 211. Out of these twenty, ten were A_1 and the remainder O or A_2. Since the A_1 character exerts an epistatic action upon the Le(b+) character on the blood

TABLE 4

ABO distribution in the different age groups of the diabetic series

	Males					Females				
Age	No.	Group O		Groups A+B+AB		Age	No.	Group O		Groups A+B+AB	
		No.	Per cent	No.	Per cent			No.	Per cent	No.	Per cent
0- 9	55	30	54.5	25	45.5	0- 9	52	30	57.7	22	42.3
10-19	107	53	49.5	54	50.5	10-19	97	48	49.5	49	50.5
20-29	95	47	49.5	48	50.5	20-29	52	25	48.1	27	51.9
30-39	83	37	44.6	46	55.4	30-39	42	17	40.5	25	59.5
40-49	88	45	51.1	43	48.9	40-49	70	29	41.4	41	58.6
50-59	56	26	46.4	30	53.6	50-59	110	46	41.8	64	58.2
60 and over	25	8	32.0	17	68.0	60 and over	60	22	36.7	38	63.3
Total	509	246		263			483	217		266	

TABLE 5

Distribution of Group O in the different age groups, subdivided according to familial predisposition

Age	Males						Age	Females					
	With family predisposition			Without family predisposition				With family predisposition			Without family predisposition		
	Total No.	Group O No.	Group O Per cent	Total No.	Group O No.	Group O Per cent		Total No.	Group O No.	Group O Per cent	Total No.	Group O No.	Group O Per cent
0- 9	27	13	48.1	28	17	60.7	0- 9	24	15	62.5	28	15	53.6
10-19	60	28	46.7	47	25	53.2	10-19	50	25	50.0	47	23	48.9
20-29	44	22	50.0	51	25	49.0	20-29	23	11	47.8	29	14	48.3
30-39	40	17	42.5	43	20	46.5	30-39	28	11	39.3	14	6	42.9
40-49	53	24	45.3	35	21	60.0	40-49	38	19	50.0	32	10	31.3
50-59	21	12	57.1	35	14	40.0	50-59	63	24	38.1	47	22	46.8
60 and over	5	3	60.0	20	5	25.0	60 and over	30	10	33.3	30	12	40.0
Total	250	119		259	127			256	115		227	102	

cells there were presumably only the ten O and A_2 samples which may have reacted with anti-Le^a due to a "cross reaction" with a "strong" Le(b+) receptor. Even after exclusion of the ten weak O and A_2 Le(a+) samples, there is still a significant difference between the diabetic series and the control series ($P = 0.02$ for both male and female diabetics).

DISCUSSION

Unlike the studies on the ABO distribution in duodenal ulcer, gastric ulcer, and gastric carcinoma with fairly consistent results from the different countries, the diabetic series show marked divergences. What is most difficult to explain is the finding of McConnell[3] and his associates that the divergence concerns particularly Group A, whereas Craig and Wang[4] and the present authors found Group O to differ most from the control series.

McConnell and his associates mention the possibility that the Group A excess may be a characteristic of a special "type" of diabetes, differing "genetically" from other forms of diabetes. If so, the Group O excess might be characteristic of another "type" of diabetes, reduced frequency of Group O without a simultaneous excess of Group A characteristic of a third "type," etc. If differences in the distribution of the ABO groups reflect differences in genetic "types" of diabetes, these different "types" must int. al. be of a different geographical distribution. The "type" characterized by the excess of Group A would in that case be very uncommon in Scotland and Denmark, but common around Liverpool and Oxford. Both McConnell and his associates as well as the present authors have shown that the distribution of ABO groups in diabetics differs significantly from the control series only in the case of the males, whereas the ABO distribution in female diabetics does not differ significantly from the control series. Then, the women, at any rate after the age of thirty, might be particularly sensitive to a special "type" of diabetes independent of the ABO system and equally widespread in the different geographic areas. This entire theory that persons of different ABO groups might possess a different sensitivity to a special "type" of diabetes is very interesting. At our present stage of knowledge, however, it must be considered a purely speculative attempt at explaining certain incongruities between the findings.

Other factors too may influence the ABO distribution in a diabetic series. If the diabetics are closely related, the material will show a variation too marked in relation to the theoretical variation determined by the binomial distribution. In our series, only ten patients were known to be related. Our diabetic series comprises all social classes and is derived from all parts of Denmark. Since the distribution of social groups and geographic areas in the diabetic and control series is not known, a divergence in the ABO distribution may occur between the two materials, if social or geographic factors influence the ABO distribution. It is not known whether this is so in Denmark.

As mentioned above, the Rh distribution did not differ significantly from the distribution in the controls. In this respect, it conforms with the Oxford series.

The difference between the total diabetic series and the control series in respect to the Lewis group, Le(a+), was highly significant. Le(a+) persons are nonsecretors of blood group substances A, B, and H which are excreted by the secretors int. al. in the saliva parallel to the occurrence of the corresponding antigen on the blood cells. This secretor function is dependent upon a gene, called the Se gene with the corresponding inactive allele se. The Lewis group on the blood cells is the result of an interaction between genes on several loci, int. al. the secretor genes. The Lewis group Le(a+) presupposes that the person concerned is homozygotic with regard to the gene se, viz., that he is a nonsecretor. Thus, it seems as if the nonsecretor status favors the development

of diabetes, independently of the ABO group.

The genetic composition of the population is probably not in equilibrium, and this applies to diabetes as well as to blood groups. It may be imagined, therefore, that in fact the blood group distributions found are "snapshots" of a dynamic process. The conflicting results from the three areas, Glasgow, Liverpool-Oxford, and Denmark, might indicate that the diabetic-genetic and blood group-genetic evolution of the population has reached different steps in the three areas. One of the factors which might be imagined to interfere with the diabetic-genetic development is insulin therapy. It must be assumed, however, that insulin therapy has been initiated at approximately the same time and with approximately the same efficacy in all three areas. Incidentally, it is so fairly recent that its influence upon the diabetic-genetic composition of the population cannot have left its mark except on the younger age groups. In this connection it is perhaps of importance that in all three materials the most marked divergences in ABO distribution as compared with the control series were found in patients whose diabetes had set in prior to the thirtieth year of age.

The results of the attempts to elucidate the genetic background of diabetes by means of blood group studies are so conflicting and so inexplicable, however, that they can only lead to the conclusion that no regularity has been demonstrated in the relationship between diabetes and the ABO blood group system.

The excess of Le(a+) group in male as well as female diabetics of the present series indicates a possible relationship between diabetes and the nonsecretor status. It must be tested, however, on new and larger series to learn whether nonsecretor status is tantamount to increased sensitivity to diabetes.

SUMMARY

A total of 992 diabetics from Denmark were studied with regard to ABO, Rh and Le(a+) groups.

Compared with the control series there was a significant excess of Group O among the male diabetics. In diabetic females, too, there was an excess of Group O, but not significant.

The distribution of Rh positive and Rh negative persons did not differ significantly from the control series.

A significant excess of Group Le(a+) was found in male as well as female diabetics. Since Le(a+) persons are nonsecretors of A, B, and H substance, these results indicate that possibly the nonsecretor status means an increased susceptibility to diabetes.

Comparison with the divergences within the ABO system found in other series showed results so conflicting that they must lead to the conclusion that so far no regularity in the relationship between diabetes and the ABO system has been demonstrated.

SUMMARIO IN INTERLINGUA

Gruppos de Sanguine e Diabete Mellite

Un total de 992 diabeticos ab omne partes de Danmark esseva studiate con respecto al gruppos de sanguine ABO, Rh, e Le(a+).

In comparation con le series de controlo, diabeticos mascule includeva un excesso de representantes del gruppo O. Inter le diabeticos feminin il etiam habeva un excesso de representantes de gruppo O, sed iste excesso non esseva statisticamente significative.

Le distribution de subjectos Rh-positive e Rh-negative inter le diabeticos non differeva significativemente ab le correspondente distribution in series de controlo.

Un excesso significative de representantes del gruppo Le(a+) esseva trovate inter le masculos si ben como inter le femininas diabetic. Viste que personas del gruppo Le(a+) es non-secretores de substantia A, B, e H, iste resultatos suggere que possibilemente le stato de non-secretor significa un augmentate susceptibilitate a diabete.

Un comparation del divergentias intra le systema ABO trovate in altere series produceva resultatos de character si contradictori que on debe concluder que al tempore presente nulle regularitate ha essite demonstrate in le relationes inter diabete e le systema ABO.

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Treatment of Diabetes with Metahexamide

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The effects of metahexamide—N-(3-amino-4-methylbenzenesulfonyl)-N'-cyclohexylurea—were studied in 104 diabetic patients for periods of up to eight months. The mode of action of this drug apparently is similar to that of tolbutamide and other sulfonylureas. Preliminary studies suggest that its hypoglycemic effect is about ten times that of tolbutamide and twice that of chlorpropamide. It is more rapidly absorbed from the gastrointestinal tract than either tolbutamide or chlorpropamide, and it is excreted much more slowly than tolbutamide but somewhat faster than chlorpropamide. The 50 per cent excretion time is about twenty-six hours. One third of the drug is excreted unchanged, while half is hydrolyzed to the 3-amino-4-methylbenzenesulfonamide derivative.[1] Recent reviews of the history and therapeutic particulars of oral therapy for diabetes[2-7] are many and obviate the need for repetition.

MATERIALS AND METHODS

Patients were selected from the wards and clinics of the cooperating hospitals and from private offices. Hepatic function studies were obtained initially in seventy-one subjects and were repeated in thirty-two of the forty-nine patients who responded to the drug and hence received protracted therapy. Patients were seen at one-week to one-month intervals, most frequently of course at the beginning of therapy, and serial determinations of blood sugar values and urine reducing-substances were obtained; relative stability of diet and weight during the trial was sought. Insulin was withdrawn abruptly in those patients who required less than 40 units of insulin daily, while dosage was reduced in stepwise fashion in those whose disease was more labile, or who took more than 40 units of insulin. The daily dosage of metahexamide was 200 mg. during the first three or four days and ranged from 50 to 200 mg. thereafter, with 100 mg. per day, given once daily, the maintenance dose in the vast majority of subjects. We considered control "good" when the "true" blood glucose value was below 130 mg./100 ml. during fasting or below 150 two hours postprandially and when an ante cibum urine specimen contained only a trace of glucose or none at all. Control was "fair" when the blood sugar level was below 150 mg./100 ml. fasting or below 180 two hours postprandially and when generally less than 0.5 per cent glucose was present in the ante cibum urine specimen. Those whose control was poorer were included with those in whom therapy was considered to have failed.

In three patients, an attempt was made to assess responsiveness by administration of a single oral dose of 500 mg. of metahexamide; the blood sugar level was observed at zero time and again after four hours; one of these patients was later placed on maintenance therapy. Of the remaining 101 patients, all but two received the usual therapeutic dose for at least one week; in these two, acute intolerance or impending acidosis made this course undesirable. In some of the milder diabetics in whom metahexamide failed initially the trial was continued for up to three months in the hope that prolonged therapy would ultimately prove effective, while those subjects for whom alternative therapy seemed imperative were treated accordingly. Of those included in the final analysis, thirty-three had previously been treated with carbutamide, tolbutamide or chlorpropamide; nineteen of these had proved primary failures (one month of treatment or less) and five of them were secondary failures (after one month or more of successful therapy). The age of onset of their diabetes was under forty years in eighteen of the patients.

RESULTS

Ten patients have been excluded from the final tabulation because the period of observation was too short, because they had been lost to follow-up, or because other factors rendered their response genuinely uninterpretable. In addition, two of the three patients given a single oral 500 mg. dose as a trial of their responsiveness had no decline in blood sugar. These patients were not treated further, although from what is known of tolbutamide

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response tests the conclusion that they would necessarily have failed clinical trial is not entirely warranted.

In forty-nine (53.4 per cent) of the remaining ninety-two patients the response was good or fair; in forty-three (46.6 per cent) treatment failed (table 1). In our experience, those individuals who failed to respond to 100 mg. only rarely responded when larger doses were employed.

TABLE 1

Clinical response to metahexamide

Response	Number	Per cent
Good	39	42.5
Fair	10	10.9
Failed	43	46.6
Total*	92	100.0

* Includes twenty-five known to be unresponsive to other sulfonylureas and eighteen with age of onset under forty years.

Of the nine patients who had previously responded to another sulfonylurea, two did not respond to metahexamide (one had been treated with tolbutamide over a year earlier; the other, case 6, had had a good response to chlorpropamide for the preceding two months). Five of the nineteen who previously had not responded to either tolbutamide or chlorpropamide were successfully maintained on metahexamide. Finally, one patient of the five secondary tolbutamide failures responded to metahexamide (case 3). The addition of metahexamide to the therapeutic regimen of one eighty-six-year-old obese diabetic permitted a reduction of her insulin dose from 85 to 15 units of NPH insulin daily.

Among the forty-three patients who did not respond to metahexamide were an elderly woman with concomitant thyrotoxicosis, a young malnourished Chinese male with pancreatitis and intestinal malabsorption, and an acromegalic man (previously described in detail)* who was well controlled on phenethylbiguanide and who two years previously had failed to respond to an oral tolbutamide test. One patient with a mild hyperadrenocortical syndrome was responsive to the drug, while another patient was well controlled on metahexamide only until she was given cortisone for unrelated disease, and then ceased to respond. Sixteen of the eighteen subjects whose diabetes had become manifest before the age of forty failed to respond to metahexamide.

SIDE EFFECTS

In tabulating the side effects of metahexamide we considered all 102 patients who received more than one dose. Side effects were few in number and generally mild. Eleven per cent of the patients had either definite or possible reactions to the compound, but only 5 per cent required discontinuance of treatment (table 2). Two patients whose blood sugar was adequately controlled complained of ill-defined malaise; this disappeared on cessation of therapy. Two others noted transient episodes of nausea that remitted after the first two or three days without withdrawal of the drug. One patient developed severe nausea and vomiting. After stopping metahexamide and resuming his usual 10 unit dosage of NPH insulin he became temporarily confused, presumably having suffered a hypoglycemic episode. Prolongation of prothrombin time from twenty-five to 100 seconds associated with hemorrhage from the oral cavity, urinary tract, and bowel occurred in one patient on long-term anticoagulant therapy with bis-hydroxycoumarin during the week in which a switch from chlorpropamide to metahexamide therapy was undertaken. Metahexamide was withdrawn, although signs of overt hepatic disease were lacking. After loss to follow-up of one month, hepatic function studies, when repeated, showed no deviation from normal. Any relationship between the drug therapy and the change in prothrombin time is necessarily speculative. On one occasion each, two patients had symptoms during the first few days of therapy that were compatible with mild hypoglycemia but which did not recur on continued treatment.

TABLE 2

Side effects of metahexamide (102 patients)

Side effect	Therapy halted	Therapy not halted
Malaise	2	
Gastrointestinal symptoms	1	2
Prolonged prothrombin time	1	
Hypoglycemia (?)		2
Hepatic dysfunction	1	2*
Total patients	5	6

* Effect transient (see text).

One patient (case 2) was found to have abnormal liver function tests [elevated alkaline phosphatase level and increased bromsulfalein (BSP) retention] after successful therapy of three months' duration. In two others (see case 5), increased BSP retention was similarly found on routine liver function tests after initiation of therapy, but with continued treatment these tests returned to normal. The remaining twenty-nine had no noted disturbance; no patients became jaundiced. It is possibly noteworthy that two patients with Laennec's

cirrhosis who had markedly abnormal baseline liver function tests did not evidence poorer function during metahexamide treatment. In one patient, markedly abnormal tests appearing during chlorpropamide therapy reverted to normal during treatment with metahexamide. Two patients developed keto-acidosis when metahexamide was substituted for insulin; one of the two required hospitalization.

The following case histories are illustrative:

Case 1. Good response in a maturity-onset diabetic: A. T., a seventy-eight-year-old obese woman, was found to have diabetes in 1955. Over the succeeding months her initial dose of 40 units of insulin was reduced to 7 units a day, during which time she had negative to trace glycosuria in morning fasting urine specimens. In the fall of 1958 her glycosuria increased to 2 per cent despite increases to 15 units of NPH insulin per day. On this regimen her fasting blood sugar (FBS) was 280 mg./100 ml. and her urine was recorded as "4 plus." Within a week after she had started on 100 mg. per day of metahexamide her glycosuria diminished to an occasional trace and her FBS ranged from 107 to 88 mg./100 ml. over a five-month period.

Case 2. Hepatic dysfunction in a maturity-onset diabetic after good therapeutic response: I.P., a sixty-eight-year-old obese, short woman with no family history of diabetes had had the disease for nearly three years. Because of a high renal threshold leading to the absence of glycosuria she had never been placed on insulin therapy, although her fasting and two-hour-postprandial blood sugars ranged from 165 to 253 mg./100 ml. (nine determinations over a two-year period). Her BSP retention after forty-five minutes was 1.5 per cent. She was started on metahexamide and maintained uneventfully on 100 mg. per day. Three weeks later her FBS was 134 mg./100 ml. and after three months it was 102 mg./100 ml. At this juncture the BSP test was repeated and the forty-five minute retention was found to have risen to 39 per cent. The alkaline phosphatase was 13 units (normal range 2 to 9), although the thymol turbidity was normal. Metahexamide treatment was discontinued. A week later her FBS had risen to 164 mg./100 ml. and after two weeks her BSP retention had fallen to 21 per cent.

Case 3. Fair response to metahexamide in a woman who was a secondary failure on tolbutamide: M.D., a sixty-three-year-old short and moderately obese woman, had had diabetes for about twenty years. She did not wish to lose weight. In 1955, while she was taking 10 units of NPH insulin daily, her postprandial blood sugars ranged from 238 to 271 mg./100 ml. and her fasting urine specimens were free of sugar. During the initial months of therapy, 1.5 gm. of tolbutamide daily brought her postprandial blood sugar down to 145 and 159 mg./100 ml., but further determinations during the subsequent year of therapy indicated a rise to fasting levels of 232 and 253 mg./100 ml. Insulin was again administered, now 16 units of NPH and 10 units of crystalline insulin daily, but her blood sugars continued to range from 224 to 266 mg./100 ml. In October 1958, her insulin dose was reduced in stepwise fashion and replaced with metahexamide, 100 mg. per day. Her fasting glucose fell slightly to 167 mg./100 ml. but over the next three months rose to 222 and 228 mg./100 ml. Her BSP retention was normal. As a test of the value of the drug, metahexamide was withdrawn. Within three days glucose began to appear in her ante cibum urine specimens, generally "4 plus" (2 per cent or more). On reinstitution of metahexamide her glycosuria again disappeared, although there was little change in her blood glucose.

Case 4. Lack of response in an adult who was a secondary failure on tolbutamide: J.V., a seventy-two-year-old man, had diabetes of three years' duration. Because the administration of NPH insulin was associated with local irritation and generalized urticaria, he was started on tolbutamide in May 1956. His postprandial blood sugar fell from 181 to 105 mg./100 ml. and he was aglycosuric. After seven months he again had glycosuria and his two-hour-postprandial blood sugar was 340 mg./100 ml. After a year without therapy he was given metahexamide, 100 mg. per day for two weeks, then 200 mg. daily for five weeks; his blood sugars remained in the range of 228 to 324 mg./100 ml. He was subsequently well controlled on 40 units of Lente insulin.

Case 5. Good response in maturity-onset diabetes with transient abnormality of hepatic function: L.S. had had diabetes for four of her forty-nine years. In the past she had taken 15 to 25 units of NPH insulin but had been in inadequate control, with blood sugars ranging from 200 to 240 mg./100 ml., although there was no glycosuria. On metahexamide her FBS declined to 112 mg./100 ml. After three weeks of therapy her BSP retention was found to be 23 per cent after forty-five minutes. There was no clinical evidence of hepatoxicity; therapy was continued and afforded her excellent control. Three months later redetermination of the BSP retention showed a decline to 7 per cent in forty-five minutes. There were no reasons other than drug therapy to explain this transient abnormality.

Case 6. Failure to control diabetes in a patient who had had an excellent response to chlorpropamide: T.P., a fifty-eight-year-old woman with diabetes of several years' duration, had in the past been treated with isoamyl biguanide and later with 16 units of NPH insulin. On the latter therapy her fasting blood glucose was 209 mg./100 ml. but she had no glycosuria. She was given chlorpropamide, 500 mg. per day, with a prompt fall in her blood sugar to a range of 119 to 129 mg./100 ml. She reported occasional episodes of nausea and vomiting directly related to ingestion of the hypoglycemic compound. Accordingly, she was taken off chlorpropamide and her blood sugar promptly returned to levels above 200 mg./100 ml. She was given 100 mg. of metahexamide for four weeks and then 200 mg. per day for twelve weeks, but her blood sugar ranged from 224 to 292 mg./100 ml. When metahexamide was withdrawn she was given 20 units of NPH insulin, but after two weeks of such therapy her postprandial blood sugar was 276 and she had 0.5 to 1 per cent glycosuria.

DISCUSSION

In most instances the diabetics subjected to therapeutic trial were older persons with mild disease, selected on the basis of the known responsiveness of this group to sulfonylurea compounds; however, we made no

particular attempt to confine an evaluation of usefulness to those prejudged most likely to succeed (see above). In the evaluation of therapy which is intrinsically unsuited to randomized application, preselection of subjects appropriate for trial serves to diminish the significance of over-all success rates. Then too, the vagaries of the natural history of diabetes mellitus are quite plaguing; exacerbations and remissions of established disease and precipitation and recompensation of recently manifest disease undermine the reliability of apparent drug responses. Simple withdrawal of sulfonylurea compounds as a test for coincidental remission may not lead to an immediate return of marked carbohydrate intolerance. Without doubt the clinical response of many patients is directly proportional to the enthusiasm of the physician, quite likely mediated by less-than-conscious adherence to heretofore neglected dietary regulation. Among such patients "secondary" failure may be more a matter of "tolerance" to the trial itself, rather than tolerance to the compound brought about by an adaptive increase in detoxification. Hurwitz and McCuiston[9] found, in their double-blind studies, that a third as many patients were improved on placebo as on tolbutamide.

While no theoretic objection can be raised to the strict standards of control suggested by Marble,[10] we have observed patients with noteworthy reduction of glycosuria and/or blood glucose who would have to be classed as therapeutic failures had we adopted such criteria. The criteria we employed correspond closely to those of Skinner and his co-workers[11] in their studies of the response to chlorpropamide.

In common with other sulfonylurea compounds, metahexamide apparently acts principally through beta-cell stimulation with a consequent increase in secretion of insulin.[5,12,13] Like its parent compounds it is almost totally ineffective in alloxanized rats and depancreatized dogs. Diminished hepatic glycogenolysis[12,14] probably contributes further to the activity of these compounds. Inhibition of insulin degradation, demonstrated in preparations containing relatively great concentrations of sulfonylureas,[15] is probably of little import at physiologic concentrations.[16]

The efficacy of metahexamide compares favorably with that of tolbutamide or chlorpropamide; our quite heterogeneous group included eighteen individuals whose age of onset of diabetes was under forty, in sixteen of whom treatment failed, and some twenty-five individuals already known to have been primary or secondary failures on another compound. While metahexamide also failed in most of the latter, its distinct success in controlling some of those known to be primarily or secondarily unresponsive to other sulfonamide derivatives makes metahexamide of definite value. The incidence of side effects in this series (5 per cent requiring discontinuance of the drug) is comparable to that observed by Iezzoni[17] in over 5,000 patients treated with chlorpropamide, although comparison with the 1.1 per cent requiring withdrawal from tolbutamide reported by Mehnert and his associates[18] probably relegates the usefulness of metahexamide to tolbutamide failures. However, the considerably larger number of instances of hepatotoxicity and jaundice reported with metahexamide as compared to chlorpropamide[19,20] suggests that its toxicity entirely precludes its usefulness as a therapeutic agent. In the light of this accumulated experience two principal pharmaceutical companies have withdrawn metahexamide from clinical trial in the United States, and therefore we have terminated our own study.

The majority of the nongastrointestinal reactions to metahexamide (skin rash, drug fever, eosinophilia, leukopenia, hemolytic anemia of the glucose-6-phosphate dehydrogenase deficient variety, thrombocytopenic purpura, and hepatic cholestasis) observed by others are of the so-called sensitivity type and, as such, are commonly considered to be unrelated to dose. However, we have reviewed data from 883 case reports of treatment with metahexamide, kindly made available to us by Dr. C. J. O'Donovan,[19] and have found that the incidence of both gastrointestinal and nongastrointestinal side reactions was more than three times as high in patients treated with more than 200 mg. per day as in those treated with lower dosages. It would seem clear that even "sensitivity" reactions require a certain threshold of stimulation. Hepatic reactions were observed eight times as frequently among the high-dose patients as in the lower-dose group (table 3). In light of these statistics,

TABLE 3

Survey of side effects in 883 patients on metahexamide*

Dose	0-200 mg.	200 + mg.
Patients, number	746	137
GI reactions, per cent	3.1	11.5
Non-GI reactions, per cent	3.3	10.2
Hepatic dysfunction		
Number	4	6
Per cent	0.5	4.4
Total per cent	6.4	21.7

* Courtesy of Dr. C. J. O'Donovan.

and in conjunction with our observation that adequate control can be achieved in most fundamentally responsive diabetics at doses of less than 200 mg. per day,

we feel that this treatment level represents a reasonable maximum.

SUMMARY AND CONCLUSIONS

1. Metahexamide — N-(3-amino-4-methylbenzenesulfonyl)-N'-cyclohexylurea — was given to 104 ambulatory diabetics and their clinical response was studied. Twenty-five of the subjects had already had an unsatisfactory response to another sulfonylurea compound, and eighteen had had a diagnosis of diabetes mellitus made before the age of forty.

2. In 53 per cent of the ninety-two who received full clinical trials the blood and urine glucose levels were adequately controlled. Metahexamide was effective in six instances of primary or secondary failure of other arylsulfonamides and permitted a reduction in insulin requirements in one other case.

3. After initial loading doses of 200 mg. per day for three or four days, maintenance was achieved in almost all patients with 100 mg. per day. Increased dosage seldom, if ever, increased the degree of control.

4. Side effects in our patients, at the dose levels we employed, were few in number and minimal in degree and included malaise, gastrointestinal reactions, and transient or sustained alteration in liver function without jaundice. Five per cent of the patients were unable to continue therapy because of probable or suspected drug intolerance.

5. A considerable number of side reactions—in particular, hepatotoxicity with or without jaundice—have been reported in the general experience with this drug. It has been shown that doses higher than 200 mg. per day are associated with a greatly increased incidence of such reactions. Therefore, while at least as effective as tolbutamide or chlorpropamide the toxicity of this new hypoglycemic compound makes it of doubtful clinical value.

SUMMARIO IN INTERLINGUA

Le Tractamento de Diabete con Metahexamido

1. Metahexamido — (N-(3-amino-4-methylbenzenesulfonyl)-N'-cyclohexylurea) — esseva administrate a 104 diabeticos ambulante. Lor responsas clinic esseva studiate. Vinti-cinque del subjectos habeva previemente habite un responsa nonsatisfacente a un altere composito de sulfonylurea. In dece-octo del patientes, le diagnose de diabete mellite habeva essite facite ante le etate de quaranta annos.

2. In 53 pro cento del novanta-duo subjectos in qui le essayos clinic esseva completate, le nivellos de glucosa in sanguine e urina esseva regulate adequatemente per le uso del droga. Metahexamido esseva efficace in sex casos de non-successo primari o secundari de altere arylsulfonamidos. In un septime caso illo rendeva possibile le reduction del dosage de insulina.

3. Post doses de attacco de 200 mg per die durante tres a quatro dies, un stato de mantenentia esseva effectuate in quasi omne le patientes con 100 mg. per die. Augmentos del dosage numquam o rarmente meliorava le grado de stabilisation.

4. In nostre patientes, al nivellos de dosage empleate, le effectos lateral esseva pauco numerose e de basse grado de intensitate. Illos includeva malaise, reactiones gastrointestinal, e transiente o continue alterationes in le function hepatic sed nulle jalnessa. Cinque pro cento del patientes non poteva continuar le curso therapeutic a causa del presentia probabile o suspicite de intolerantia a metahexamido.

5. Un considerabile numero de reactiones lateral—particularmente hepatotoxicitate con o sin jalnessa—se trova reportate in le litteratura que es concernite con iste droga. Il ha essite demonstrate que doses de plus que 200 mg per die es associate con altemente accrescite incidentias de tal reactiones. Ergo, ben que iste nove composito hypoglycemic es tanto efficace como tolbutamido o chlorpromazido, su toxicitate rende dubitose su utilitate clinic.

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Additional suggestive evidence for the presence of a dietary factor which influences the rate of removal of glucose from the blood has been presented by K. Schwarz and W. Mertz (*Arch. Biochem. and Biophys.* 72:515, 1957). They used male Sprague-Dawley rats weighing about 150 gm. These animals had been maintained on a 30 per cent Torula yeast ration supplemented with 50 mg. per cent DL-γ-tocopheryl acetate. The latter was used to prevent the dietary liver necrosis produced by the above diet (*Nutrition Reviews 16*:149, 1958).

When 125 mg. of glucose were given per 100 gm. of body weight, Mertz and Schwarz (*Arch. Biochem. and Biophys. 58*:504, 1955), found that the removal of excess blood glucose occurred at the rate of 2.4 per cent per minute. When similar experiments were carried out on rats fed the McCollum lactating diet (containing ground whole wheat, whole milk powder, corn oil, casein and salts), the removal rate was 4.2 per cent. A purified casein-sucrose diet containing all the ingredients necessary to produce rapid increases in body weights of rats resulted in a glucose removal rate of 1.5 per cent.

Schwarz and Mertz (*loc. cit.*) were able to produce a concentrate from defatted pork kidney powder which, when given as a single dose by stomach tube at the rate of 100 μg. per 100 gm. of body weight, increased the blood glucose removal rate of the rats fed the vitamin E supplemented Torula yeast ration from 2.2 per cent to 4.5 per cent. The response was apparent eighteen hours after giving the concentrate by stomach tube. The substance responsible for the above effect (designated the Glucose Tolerance Factor) differs from the Factor 3 of Schwarz (*Nutrition Reviews, op. cit.*) since Schwarz and Mertz (*loc. cit.*) noted that neither inorganic selenium nor highly purified Factor 3 concentrates had any effect on the rate of removal of glucose from the blood. Both of the latter are effective in preventing or curing the dietary liver necrosis which develops when rats are fed the unsupplemented Torula yeast ration.

A recent report by these investigators (W. Mertz and K. Schwarz, *Fed. Proc.* 17:1, 1958) indicates that a number of commercial stock diets produce low blood glucose removal rates.

The significance of the above observations still has to be established. It is difficult to assess the influence of the differences in blood sugar levels following a dose of glucose upon other physiological functions. Whether these dietary factors will ever become important is questionable at the moment since rats can grow and reproduce on rations that appear to be deficient in the postulated factor(s).

From *Nutrition Reviews*, Vol. 17, No. 2, pp. 50-52, February 1959.

Morphology and Cytochemistry of Alpha Cells of the Rabbit Pancreas

Effects of Glucagon, Insulin and Infusions of Glucose

John Logothetopoulos, M.D., and James M. Salter, Ph.D., Toronto

Since there is considerable evidence that glucagon originates in the alpha cells of the pancreatic islets, it might be expected that the administration of glucagon would produce a compensatory atrophy of these cells. Kracht[5] has reported that daily injections of glucagon reduce the size of the nuclei of alpha cells in rabbit and rat pancreas, but he did not describe any cytoplasmic changes.

It was reported from this laboratory[3] that force-fed rats could be made temporarily diabetic by the administration of large amounts of glucagon. Microscopic examination of the pancreases from these animals revealed definite degranulation of the beta cells and degranulation and atrophy of the acinar cells. The appearance of the alpha cells suggested that they were undergoing some involutionary process but similar changes were evident in the cells of other tissues such as the exocrine pancreas and liver. A detailed histological and cytochemical study on rabbits treated with glucagon was next undertaken. In this species two methods have proved extremely useful in studying alpha cells in unfixed pancreas. These are: (1) dark field illumination microscopy of fresh frozen sections mounted in glycerin and (2) visualization of zinc in alpha cells by intravital chelation with dithizone after complete depletion of the zinc of the beta cells by previous glucose injections.

While this work was in progress Lazarus and Volk[6] reported the production of atrophy of alpha cells in rabbits after prolonged treatment with glucagon. The results of our investigations confirm and extend their findings. Evidence strongly indicating that the effect on alpha cells is specific and that atrophy of alpha cells is well advanced after one week of glucagon treatment will be presented. The results indicate also that the morphology of alpha cells is not affected by intravenous infusion of large amounts of glucose for four weeks or by prolonged treatment with insulin.

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METHODS AND PROCEDURES

Rabbits. Young adult male albinos weighing 2.8 to 3.3 kg. were used. Under nembutal anesthesia a midline abdominal incision was made and a polyethylene tube was inserted into the stomach (about 7 to 8 cm. from the pylorus). The tube was fixed by two purse-string sutures at the site of insertion and by three separate inverting sutures along its course on the anterior stomach wall. The tube, after piercing the lateral abdominal wall just below the left costal margin, was channelled subcutaneously to a small incision at the base of the left ear. The end, which protruded 2 to 3 cm., was stoppered with a short glass rod. The rabbits were allowed to recover for fifteen days before they were used.

Histological technics. The rabbits were killed by an intravenous injection of nembutal. Portions of the splenic and duodenal part of the pancreas were fixed in Zenker-Formol solution (ten hours), Bouin's-Allen fluid (twenty-four hours) and in 10 per cent formalin with 2 per cent calcium acetate (seven hours). As quickly as possible after killing the animals, frozen sections were cut at 7.5 μ from unfixed samples of the pancreas and mounted in glycerin.

Paraffin sections from pancreas fixed in Zenker-Formol and Bouin's-Allen fluids were stained with hemalum-eosin, aldehyde-fuchsin-trichrome,[2] Gomori's chromalum hematoxylin,[3] and Periodic Acid Schiff. The histochemical method for tryptophan developed by Glenner and Lillie[4] was applied to the formalin fixed material. It gives a positive blue staining of the granules of the alpha cells, probably due to the high tryptophan content of glucagon (Levine and Glenner).[7]

The fresh frozen sections mounted in glycerin show that the alpha cells of the islets of the rabbit have a silvery-white cytoplasmic granulation when examined under dark-field illumination. Only the nuclei of beta

cells and of the acinar cells show a weak yellowish-white luminescence (figure 6).

The livers of all the animals were fixed in Gendre's fluid and formol calcium. Paraffin sections were stained with hemalum-eosin and Periodic Acid Schiff. Frozen sections were stained with Oil Red-O. Specimens from the kidney, heart, submaxillary, salivary gland, parotid, testis, thymus, adrenal, pituitary, stomach, duodenum and jejunum of the glucagon-treated and control animals were examined histologically. These were fixed in formol-calcium, Helly's and Gendre's fluids. Paraffin sections were stained with hemalum-eosin and Periodic Acid Schiff. A Mallory trichrome method[1] was used for sections of the pituitary gland and the silver reduction method of Mason for sections from the stomach and intestine.

Intravital zinc-chelation reaction. After an intravenous injection of diphenylthiocarbazone (dithizone), the islets of the rabbit appear as intense red-purplish spots, and contain violet-red granules of zinc-dithizonate as demonstrated and studied by Wolff et al.[9,10]

In our series of experiments, an alkaline solution of dithizone (200 mg./kg. B.W.) was slowly infused for forty-five minutes. Fresh frozen, 7.5 μ sections, were prepared with a cold-knife technic; mounted in glycerin; and examined under dark-field illumination with visible light. Alpha and beta cells in normal animals are found to be filled with intensely orange-colored zinc-dithizonate granules and are indistinguishable. However, when complete depletion of reactive zinc from the beta cells is achieved by intravenous infusions of glucose (25 ml. of a 20 per cent glucose solution six times daily) for three to four days before the dithizone infusions, only the alpha cells are filled with orange granules. Their identity is confirmed easily because the zinc-dithizonate granules fade out in the fresh frozen sections (within six to twenty-four hours) and the natural silvery-white granulation of the alpha cells becomes evident under dark-field illumination.

Glucagon-treated rabbits. The seven controls and the nine treated animals were fed at 9:00 a.m. and at 1:00, 6:00 and 11:00 p.m. Each feeding consisted of 25 gm. of finely ground rabbit chow and 5 gm. of dextrin made into a thin paste with water. This mixture was forced slowly through the indwelling stomach tube with a modified 100 cc. glass syringe.

After each feeding the animals received either a subcutaneous injection of glucagon* suspended in isotonic saline or of the saline alone. A total of 7 mg. of glucagon was given daily by three injections of 1.5 mg. and one of 2.5 mg. (11:00 p.m.). Total urine was collected daily and tested for reducing substances. Blood samples for glucose estimation were taken once a week from the animals kept for twenty-four days, at one, two and one-half and four hours after the morning injection of glucagon.

Three glucagon-treated animals and two controls were sacrificed on the eighth day. Two treated and two control animals were sacrificed on the fifteenth day and four treated and four controls on the twenty-fourth day.

An additional four rabbits were injected with glucagon and fed as described above, but they received glucose intravenously (25 cc. of a 20 per cent solution six times daily) for the last three days of the experimental period, and an infusion of dithizone immediately before death. Two of these animals were sacrificed on the eighth and two on the sixteenth day of treatment.

Glucose-infused and insulin-treated rabbits. Five tube-fed rabbits received intravenous injections of glucose daily through an indwelling siliconed polyethylene tube inserted through the external jugular vein into the superior vena cava. Twenty-five milliliters of a 20 per cent glucose solution were administered four times daily. The tube was filled with a weak heparin solution after each infusion.

All of these rabbits were killed between the fifteenth and twenty-fifth day. Two of them received an intravenous infusion of dithizone before sacrifice. The histology of the islets was studied using the methods described.

Protamine-zinc insulin (8 to 10 U.) was administered subcutaneously to six rabbits daily. Food was restricted to about two thirds of the amount normally consumed by such rabbits, and was given in small portions throughout the day. Two of the rabbits died during hypoglycemic attacks. The remaining four were sacrificed after two to two-and-one-half months of treatment.

RESULTS

Glucagon-treated rabbits

The glucagon-treated tube-fed rabbits excreted ½ to 15 gm. of sugar daily in their urine. The highest blood sugar values were reached two to two and one-half hours after glucagon was given and the values returned to normal levels three to five hours after the injection. The weight of the controls increased during the experimental periods while the weight of the treated animals either remained constant or decreased slightly.

Islet Histology. Hypertrophy and degranulation of the beta cells, with occasional mitotic figures, were evident in the animals killed eight days after starting the in-

*Crystalline Glucagon—Lilly—Lot No. 258-234B-54-2.

jections of glucagon. These changes were much more pronounced in the animals maintained on glucagon for fifteen and twenty-four days. Most of the ductule-like structures found in these hyperplastic islets, on serial sectioning, were found to result from follicle-like arrangements of the beta cells. Vacuolation and glycogen deposition were not seen in the beta cells or in the lining cells of the ducts.

There was a definite change in the alpha cells after eight days of glucagon treatment. Their nuclei were smaller, and showed a denser chromatin pattern and smaller nucleoli. A diminution of the cytoplasm was also present. This was most strikingly demonstrable in the fresh frozen sections examined with dark-field illumination. In stained paraffin sections no alpha cells were found showing a definite paranuclear macula and a negative Golgi image. A great proportion of the alpha cells in the control animals showed this characteristic appearance (figure 1).

Clusters of alpha cells found in larger islets seemed to consist mostly of nuclei with little cytoplasm between them.

The histochemical reaction for tryptophan remained positive in the reduced cytoplasm. Zinc-dithizonate granules were produced by the intravenous injection of dithizone. The atrophic changes of the alpha cells were much more advanced on the fifteenth and twenty-fourth day of glucagon treatment. Definite silvery-white alpha cells were absent from the fresh frozen sections examined by dark-field illumination. Islets had to be distinguished from acinar tissue by the pattern of their constituent cells. A faintly whitish and narrow perinuclear halo or a dense clustering of small nuclei at the periphery of an islet were the criteria for identification of alpha cells (figures 8 and 9). Reactive zinc could still be demonstrated in many alpha cells in which a cytoplasmic luminescence could scarcely be seen (figures 7 and 8).

In fixed and stained sections one could hardly distinguish any alpha cells with low magnification. At higher magnifications the alpha cells consisted of hyperchromatic nuclei surrounded by poorly stained, extremely shrunken cytoplasm (figures 2 and 3). In very few of them (figure 5) a positive reaction for tryptophan could be shown. The impression was gained that the absolute number of alpha cells might also be reduced. Since in many fields there was difficulty in distinguishing them with certainty from marginal acinar cells and endothelial cells an estimation of the alpha/beta ratio would have been very unreliable. Because of the hyperplastic and hypertrophic changes in the beta cells the significance of this ratio cannot be assessed properly without an evaluation of islet mass and cell mass.

Histology of other tissues. The comparative study of the histology of other tissues of the controls and of the rabbits treated for twenty-four days with glucagon, did not reveal any differences. No other cellular structures were found to be undergoing an atrophic change. Glycogen was present in the livers and in the distal tubules and loops of Henle of the kidneys of the glucagon-treated animals only. Zymogen granules and basal hematoxylinophilia were equally preserved in the acini

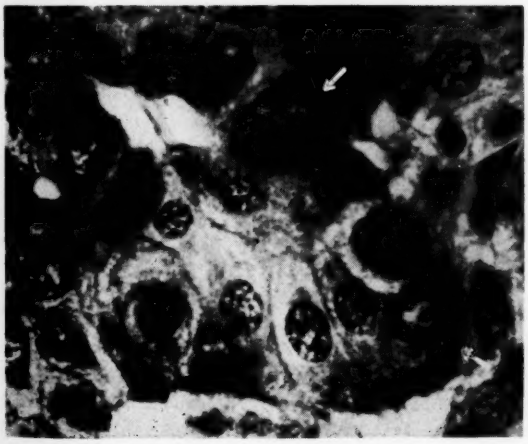


FIG. 1. Peripheral portion of pancreatic islet of control rabbit. Active α-cells (dark) with broad granulated cytoplasm, paranuclear macula and negative Golgi image. Gomori's chrome alum hematoxylin, X 775.

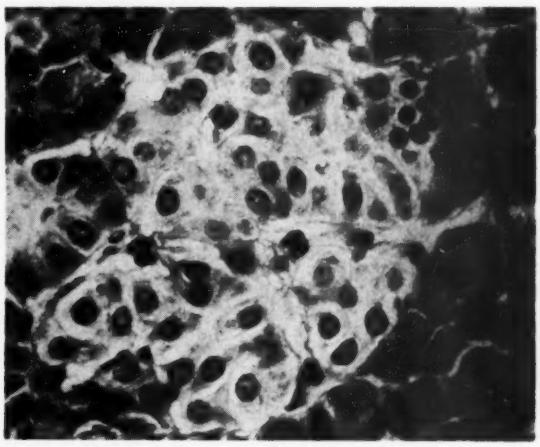


FIG. 2. Pancreatic islet of rabbit treated with glucagon for twenty-four days. A group of five atrophied α-cells at the upper right corner. Gomori's chrome alum hematoxylin, X 340.

of the pancreas and salivary glands. There were no differences in the weights of the thyroid glands, testes, adrenals or pituitary glands in the animals of the two groups, and no distinction could be made in the histological appearance of the glands of control and glucagon-treated animals. Spermatogenesis and the number of mitoses in the thymus were identical in both groups. Fully granulated, silver-reducing cells were present along the intestinal mucosa of both treated and control animals.

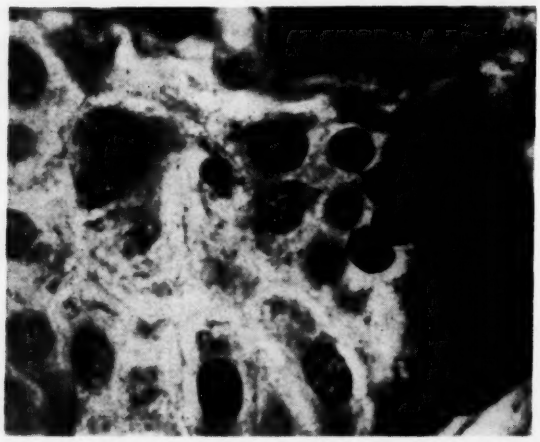


FIG. 3. High power view of upper right portion of islet of figure 2. X 775.

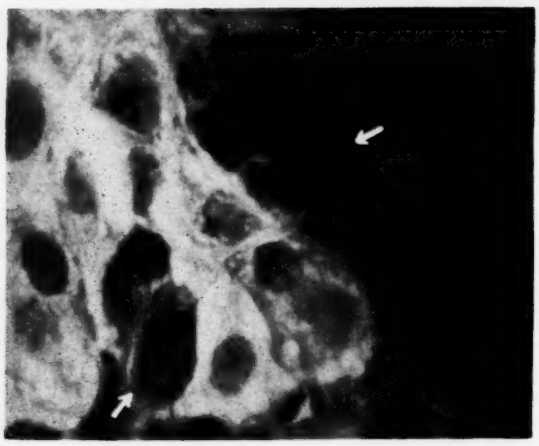


FIG. 4. Pancreatic islet of control rabbit force-fed for twenty-four days. Six α-cells at the periphery of an islet with a strong reaction for tryptophan. Postcoupled benzylidine reaction, X 775.

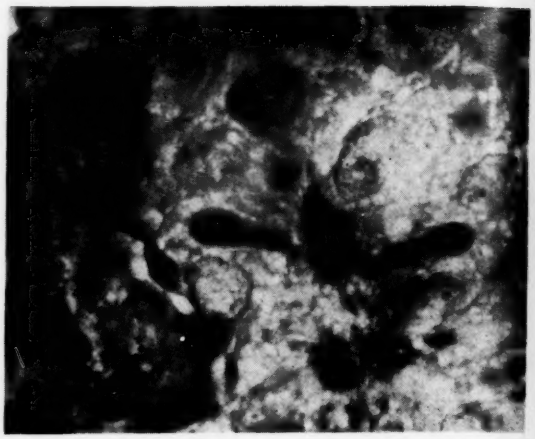


FIG. 5. Pancreatic islet of force-fed rabbit treated with glucagon for twenty-four days. Two α-cells with a positive reaction for tryptophan in their shrunken cytoplasm. Compare their nuclei with the nuclei of the neighboring β-cells. Postcoupled benzylidene reaction, X 775.

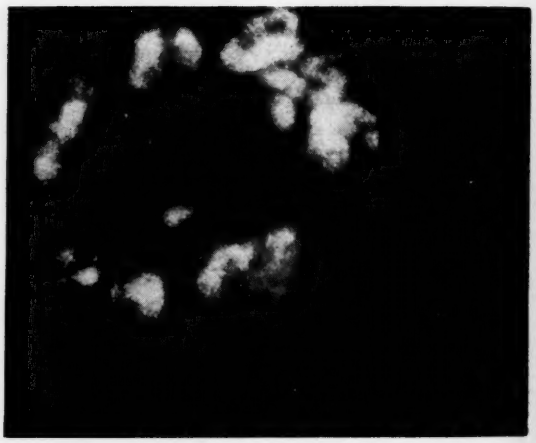


FIG. 6. Pancreatic islet of control rabbit. Fresh-frozen section. Dark field illumination. Natural luminescence of cytoplasmic granules of α-cells. X 270.

Glucose-infused rabbits

Hypertrophy, hyperplasia and degranulation of the beta cells were, on the whole, much the same as in the glucagon-treated rabbits. Reactive zinc as judged by intravital chelation was totally absent. The alpha cells were very well preserved, with broad, fully granulated cytoplasmic rims. They contained large amounts of reactive zinc, as shown by the intravital chelation reaction (figures 11 and 12). Their cytological appearance did not differ from the alpha cells of untreated controls.

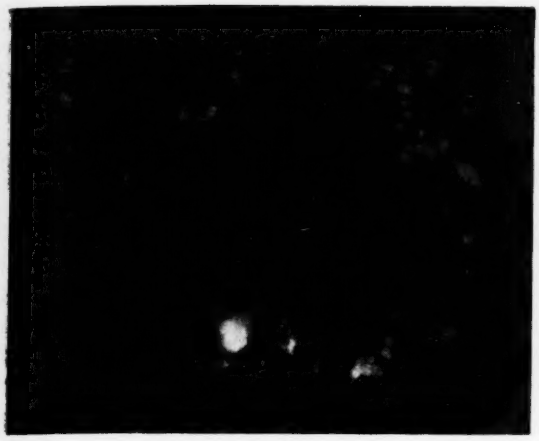


FIG. 7. Pancreatic islet of rabbit treated with glucagon for seventeen days injected with dithizone before sacrifice. Fresh-frozen section mounted in glycerin. Complete lack of precipitate of zinc-dithizonate in the β-cells. White zinc-dithizonate granules (natural luminescence orange) in three groups of densely packed small α-cells. Dark field illumination, X 180.



FIG. 9. Pancreatic islet of rabbit treated with glucagon for twenty-four days. Fresh-frozen section. Dark field illumination. Practically complete absence of cytoplasmic luminescence between the aggregate of small α-cell nuclei at the periphery of the islet. X 270.

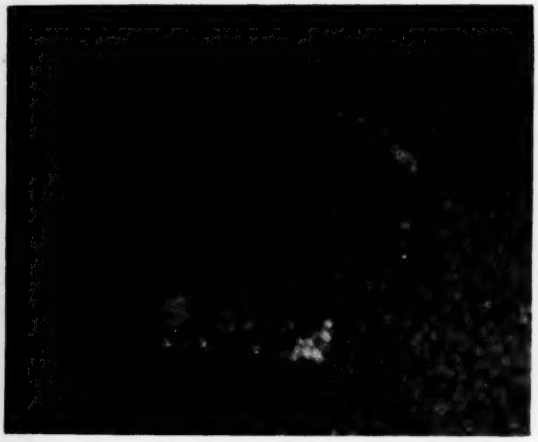


FIG. 8. Same section of figure 7, ten hours later. Zinc-dithizonate granules have dissolved away. Very little cytoplasmic luminescence between the densely packed nuclei of the α-cells. Dark field illumination, X 180.

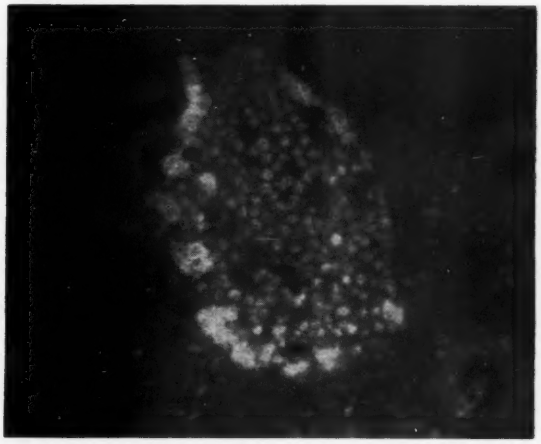


FIG. 10. Pancreatic islet of rabbit treated for 2½ months with insulin. β-cells nuclei crowded. Well preserved α-cells at the periphery. Fresh-frozen section. Dark field illumination, X 180.

Insulin-injected rabbits

The islets appeared to be smaller than those of control animals. There was a reduction in the size of the beta cells and in their cytoplasmic/nuclear ratio. While their granulation and content of reactive zinc were appreciable these were less than in the controls (figure 10).

The alpha cells did not show any obvious change in size, granulation or zinc content.

DISCUSSION

The atrophic changes of the alpha cells elicited by the administration of glucagon developed within the first week of treatment and progressed rapidly. Nuclear and cytoplasmic shrinking, with poor granulation and decreased content of reactive zinc, was fully developed on the twentieth to twenty-fourth day of treatment. The normal cytological appearance of a great number

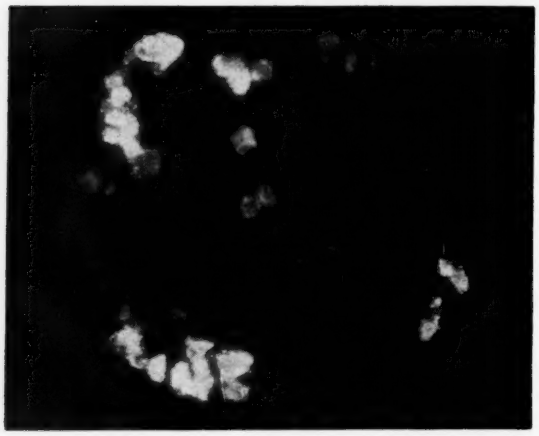


FIG. 11. Pancreatic islet of force-fed rabbit injected with glucose intravenously four times daily for twenty-two days. Dithizone injection before sacrifice. Fresh-frozen sections mounted in glycerin. Heavy white precipitate of zinc-dithizonate (orange in original preparation) superimposed on the white-silvery luminescence of granulated cytoplasm of the α-cells. Complete absence of zinc in the β-cells. Dark field illumination, X 180.

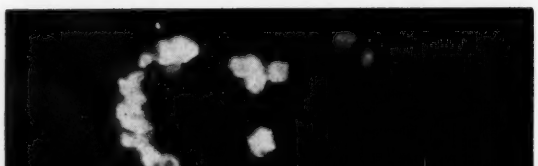


FIG. 12. Same section as figure 10, fifteen hours later. Zinc-dithizonate granules have dissolved away. Natural luminescence of the α-cell granulation prominent. Compare with glucagon treated rabbits of figures 8 and 9. Dark field illumination, X 180.

of other tissues and endocrine glands makes it highly improbable that one is dealing with a non-specific effect of high doses of glucagon. The best supported interpretation of the findings is that a specific atrophy of alpha cells is occurring, which is the result of administration of the hormone which they produce.

The dose of glucagon administered subcutaneously (7 mg.) was extremely high, when compared with the single dose needed to produce a hyperglycemic response, but it was chosen in order to achieve an adequate level throughout the twenty-four hours. Continuous intravenous infusion of smaller amounts of glucagon might have been equally effective.

The elucidation of the mechanism of the atrophy would necessitate further experimental work. Multiple intravenous glucose infusions, reproducing the pattern of the hyperglycemia that follows injections of glucagon, did induce, within the same time interval, a marked hyperplasia and degranulation of the beta cells. Alpha cells remained active, fully granulated and with a normal zinc content. Prolonged treatment with insulin (two to two and one-half months) with restriction of caloric intake, also failed to induce a change of the alpha cells. Neither atrophy attributable to underactivity, nor degranulation attributable to overactivity was seen.

The question whether the atrophy of the alpha cells is produced by an inhibition of formation of a trophic hormone or whether it is a direct response to a change in the concentration of a metabolic product remains unanswered.

SUMMARY

1. The alpha cells of the pancreas of rabbits undergo a progressive atrophy during treatment of the animals with glucagon.

2. Nuclear and cytoplasmic shrinkage with loss of granulation was fully established after twenty to twenty-five days of treatment.

3. The involution of the alpha cells is considered a specific effect of glucagon because no cellular structure in other endocrine glands or in a number of different tissues showed similar changes.

4. Prolonged treatment with insulin and multiple infusions of glucose did not affect the alpha cells.

5. Two new cytological methods are described for the evaluation of the granulation and of the zinc content of the alpha cells in unfixed pancreas.

SUMMARIO IN INTERLINGUA

Le Effectos De Glucagon, De Insulina, E De Infusiones De Glucosa In Le Morphologia E Cytochimia Del Cellulas Alpha In Le Pancreas De Conilios

1. Le cellulas alpha del pancreas de conilios suffre un atrophia progressive durante le tractamento del animales con glucagon.

2. Contraction nucleari e cytoplasmic con perdita de granulation esseva plenmente establite post vinti a vinticinque dies de tractamento.

3. Es opinate que le involution del cellulas alpha es un effecto specific de glucagon, proque nulle structura cellular in altere glandulas endocrin o in varie altere organos exhibiva simile alterationes.

4. Prolongate cursos de tractamento con insulina e multiple infusiones de glucosa non afficeva le cellulas alpha.

5. Es describite duo nove methodos cytologic pro le evalutation del granulation e del contento de zinc del cellulas alpha in le pancreas non fixate.

ACKNOWLEDGMENT

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The authors are grateful to Professor C. H. Best for his invaluable criticism and advice.

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J. A. Uram, L. Friedman and O. L. Kline (*Resumes des Communications, 4th International Congress of Nutrition, p. 272, Paris 1957*) initiated their studies on activity of pancreatic digestion in rats by measuring the blood sugar levels following a dose of starch given by stomach tube. The marked differences in the blood sugar levels in animals maintained on different diets suggested that the increase in the blood sugar level was related to the previous dietary history of the animal.

As an extension of these preliminary studies, Uram, Friedman and Kline (*Am. J. Physiol.* 193:521, 1958) found that the level of fat and sucrose in a purified diet had practically no effect on the height of the blood sugar curve following an oral dose. This was true when the percentage of calories in the diet from sucrose and fat were in diet A, 25 and 53 and in Diet B, 66 and 12, respectively. In both cases, the rats had been starved for twenty-four hours and then were given by stomach tube a 40 per cent glucose solution at the rate of 4 gm. per kg. of body weight. The maximum blood glucose levels in the rats fed the above diets were 160 to 180 mg. per 100 ml. of blood one hour after giving the glucose dose. Rats fed a stock diet composed of natural products showed maximal blood sugar levels of 110 mg. (in the two-hour blood sample) under similar circumstances. Four hours after the dose was given, the blood glucose concentration in all rats was approximately 75 mg. per cent.

Uram, Friedman and Kline (*loc. cit.*) believe their work suggests the presence of a dietary factor which increases the rate of removal of sugar from the blood following an oral glucose tolerance. This is based on the following: The high glucose tolerance curve of the rats fed the purified diet could be reduced by feeding the stock diet for two weeks. Since the blood glucose levels in all groups at the end of four hours is approximately the same, the investigators concluded that there were no differences in the rate of absorption of glucose from the gastrointestinal tract.

From *Nutrition Reviews*, Vol. 17, No. 2, pp. 50-52, February 1959.

Effect of Growth Hormone on the Pancreas and Adrenals of Rabbits

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After hypophysectomy the pancreatic islets of the rat cease to grow, but show no appreciable atrophy.[1] The inference is an absence of any tropic control of the islets by the pituitary. Crude anterior pituitary extracts, nevertheless, exert a hyperplastic influence on the pancreatic islets of the rat,[2-5] mouse,[6] dog,[7-9] rabbit[10-13] and newt.[14] This investigation was undertaken to determine the effect of the growth hormone on the islets of the rabbit. During its course interesting relations were noted between the hormone and adrenals.

MATERIALS AND METHODS

Purified growth hormone supplied by the Armour and Organon Laboratories was made into a 0.3-0.5 per cent solution with sterile normal saline at *p*H 8, immediately transferred to 1 cc. ampoules and frozen, and only thawed out just before use. The solution was given subcutaneously with aseptic precautions to six male and seven female English rabbits weighing between 2,010 gm. and 3,070 gm. The animals were kept in metabolism cages, allowed to eat ad lib of a pellet diet prepared by the Associated London Flour Millers, Ltd., and placed under no restrictions as regards water supply. The periods of control before and after treatment with growth hormone lasted ten to twenty-nine days and five to eleven days respectively. The body weight, calorie intake, nitrogen balance and urine volume and specific gravity were measured daily, while the blood and urine sugar levels were determined as necessary. The nitrogen content of the diet, urine and stool was estimated by the Kjeldahl method, blood sugar by the Hagedorn-Jensen method, and urine sugar by Benedict's quantitative method. The insulin sensitivity was tested by determining the blood sugar before and at five- or ten-minute intervals for sixty minutes after the intravenous injection of 0.5 unit of insulin.

The pancreas of each animal was dissected out immediately after death, fixed in Helly-Zenker solution, embedded and cut in paraffin, and stained with alcoholic eosin and hematoxylin and by Gomori's chrome alum hematoxylin phloxin method. The size of the islets was determined at a magnification of 200 by means of a calibrated 0.5 mm. squared grid inserted into the ocular of the microscope. Measurement was made of 225 islets in four randomly selected groups of eighteen or nineteen islets in the head, body and tail of each pancreas. The percentages of α and β cells in the islet tissue were assessed by examining a total of 2,000-2,500 cells in each pancreas.

Six male and seven female English rabbits weighing between 2,135 gm. and 3,020 gm. served as controls. They were individually matched with the injected group in respect to sex, body weight and duration on pellet diet* and were kept under the same experimental conditions.

RESULTS

The data as regards growth hormone, body weight, glycosuria and blood sugar are assembled in table 1 and illustrated in figures 1—4.

Glycosuria. Nine of the thirteen rabbits showed glycosuria. This feature was noted in five of nine rabbits injected with constant doses of hormone, in all three animals given increasing amounts of hormone, and in rabbit 58. In the group treated with constant doses of hormone, glycosuria appeared on the third to the tenth day, lasted from five days consecutively to twenty days intermittently, and reached a peak of between 2.3 gm. and 14.1 gm. per 24 hr. In the series given increasing amounts of hormone, glycosuria showed on the ninth to the thirty-fifth day, continued from two days to thirteen days intermittently, and attained a maximum of between 1 gm. and 3 gm. per 24 hr. In rabbit 58, glycosuria was noted on the third and thirtieth days, lasted one day on the first occasion and eleven days intermittently on the second, and reached a peak of 2.1 gm. per 24 hr. during the latter period. Glycosuria occurred with equal frequency in the male and female rabbits, while the Armour hormone and the Organon hormone appeared to be equally effective in eliciting it. Concurrent with the glycosuria five

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*The pellet diet consisted of: 15 per cent wheat feed, 20 per cent barley meal, 30 per cent grass meal, 8 per cent meat and bone meal, 15 per cent ground nut meal, 1 per cent salt, 10 per cent linseed cake, 1 per cent chalk.

TABLE 1

Rabbit	Sex	Growth hormone: Daily amount* (mg.)	Growth hormone: Duration (days)	Growth hormone: Total (mg.)	Body weight (gm.): At start of Experiment	At start of Treatment	At end of Treatment	At end of Experiment	Glycosuria: Day of appearance	Glycosuria: Duration (days)	Glycosuria: Maximum (gm. per 24 hr.)	Blood sugar (mg. per cent): Before treatment	Blood sugar (mg. per cent): During glycosuria	Blood sugar (mg. per cent): After treatment
51	F	4 A	20	80	2,385	2,315	2,415	2,375	—	—	—	152	—	—
52	F	12 A	22	264	2,470	2,425	2,500	2,380	7	14	5.25	133	142,118,118	103
53	M	11 A	15	165	2,730	2,780	2,800	2,865	8	5	2.99	110	108	—
54	F	13 A	15	195	3,070	3,020	2,965	2,830	—	—	—	155	—	—
55	F	14 A	22	308	2,235	2,300	2,530	2,410	7	14	14.12	102	146,187	96
56	F	14 A	22	311	2,000	1,955	2,085	2,010	10	8	11.01	121	176,226,217	138
57	M	5-30 O	30	525	2,445	2,400	2,520	2,410	28	3	1.03	111	108	104
58	F	16-8-4 O	72	352	2,180	2,235	2,420	2,525	3&30	1&11	1.2&2.05	112	98	97
59	M	5 O	70	350	2,100	2,145	2,535	2,475	—	—	—	140	—	149
60	F	5-25 A	35	525	2,575	2,605	2,685	2,695	35	2	1.42	133	120	117
61	M	15 A	26	390	2,470	2,585	2,850	2,805	3	20	2.33	149	158	133
62	M	15 O	22	326	2,405	2,395	2,620	2,595	—	—	—	134	—	113
63	M	10-20 O	30	450	2,640	2,720	2,920	2,875	9	13	3.04	114	138	126

Notes: 1. The initial dose of 16 mg. daily in rabbit 58 produced such an acute loss of appetite as necessitated reduction of the hormone on the sixth day to 8 mg. and on the seventh to 4 mg., at which level the daily dose was thereafter maintained.
2. The daily amount of hormone in rabbits 57, 60 and 63 was increased by 5 mg. at intervals of five days or more.
3. The glycosuria in rabbits 52, 55, 58, 61 and 63 was intermittent.
*"A" and "O" signify Armonur and Organon hormone respectively

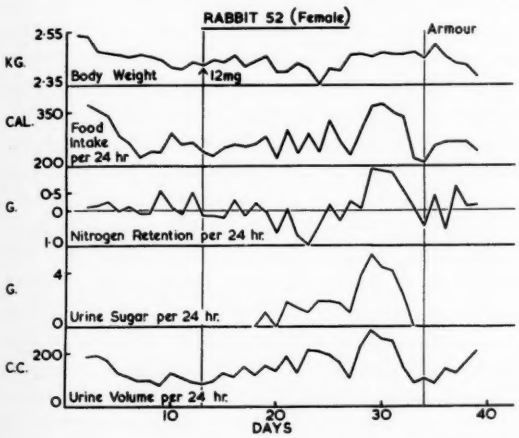


FIG. 1. Rabbit 52 received 12 mg. growth hormone daily for twenty-two days (between vertical lines).

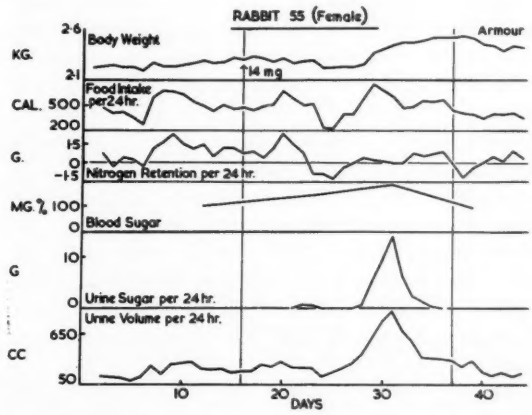


FIG. 2. Rabbit 55 was given 14 mg. growth hormone daily for twenty-two days (between vertical lines).

of the nine rabbits had a normal blood sugar at the time of testing (10 a.m.) whereas the others exhibited a slight to moderate hyperglycemia. No ketonuria was ever observed.

Food intake, nitrogen balance and body weight. The growth hormone had a variable effect upon the appetite of the animals. Thus, in some an initial fall in food intake was followed by a rise to above normal whereas in others an initial increase in food intake was succeeded by a fall to subnormal (figures 1, 2 and 4). The hormone also induced increased nitrogen retention on a normal or even subnormal food intake and incidentally led to replacement of a falling by a rising body weight or an accelerated rate of increase in body weight (figure 3). These effects were more or less reversed during the severer glycosuric periods, but were occasionally restored, despite persistence of the glycosuria, through increase of the appetite to above normal (figure 1). Withdrawal of the hormone was sometimes followed by a decline in nitrogen retention and body weight in spite of a sustained or increasing food intake (figure 3).

Urine volume. Assessment of this factor requires prior mention of two items. First, the urine volume of the animals, while they were being controlled, varied more or less directly with the food intake; and second, polyuria is a feature of glycosuria. During these experiments the growth hormone induced an increase in urine volume in eleven of the thirteen rabbits (table 2). In the responsive animals the polyuria ranged on the

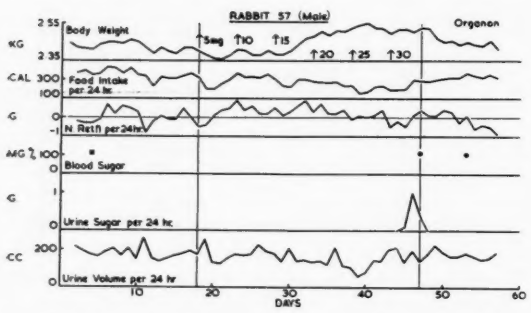


FIG. 3. Rabbit 57 received 5-30 mg. growth hormone daily for thirty days (between vertical lines).

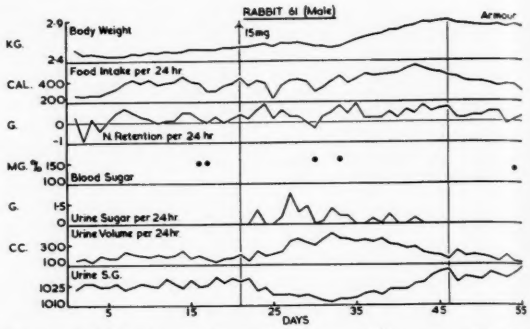


FIG. 4. Rabbit 61 was given 15 mg. growth hormone daily for twenty-six days (between vertical lines).

average from 10 per cent to 118 per cent above the control level and was more than 20 per cent greater than the normal in nine cases. The increase in urine volume was commensurate with an increase in food intake in two animals, but in the others the food intake at the time was either reduced or normal or increased to a degree relatively much less than the increase in urine volume. Any associated glycosuria, moreover, was at most only moderate in amount and could not account for the increase in urine volume, even in association with an augmented food intake. The largest daily polyurias occurred in rabbit 55—food intake + 19 per cent, urine volume + 268 per cent, urine sugar 14.1 gm. per 24 hr. (figure 2), and rabbit 58—food intake + 34 per cent, urine volume + 182 per cent, no glycosuria. The specific gravity of the urine in two of the polyuric animals was reduced, especially in the more polyuric rabbit (figure 4).

Insulin sensitivity. The seven rabbits tested gave results as in table 3. The means of the maximum falls in blood sugar of the seven animals before and after treatment were 42 and 45 mg. per cent respectively. During treatment the blood sugar in rabbits without glycosuria showed a mean maximum fall of 25 mg. per cent, while the mean maximum fall in blood sugar for animals with glycosuria was 12 mg. per cent. Insulin sensitivity was thus substantially reduced throughout administration of the hormone, particularly during periods of glycosuria. Again, resistance to the hypoglycemic action of injected insulin developed early in treatment and remained constant until a further increase occurred with the appearance of sugar in the urine. Glycosuria having disappeared, however, insulin sensitivity improved to a grade relatively greater than obtained prior to the glycosuria. Further, a small dose of hormone was as efficient as a large dose in inducing insulin resistance so that this phenomenon appeared to depend more upon the sensitivity of the animal to the action of the hormone than upon the size of the dose.

Organ measurements. The mean weights of the heart,

TABLE 2

Relation of urine volume to food intake and glycosuria

Rabbit	Period of treatment analyzed (day-day)	Mean food intake (cals. per 24 hr.) Control	Treatment with G.H.	Percentage increase or decrease	Mean urine volume (cc. per 24 hr.) Control	Treatment with G.H.	Percentage increase or decrease	Maximum glycosuria (gm. per 24 hr.)
51	11-16	223	359	+61	133	205	+ 54	—
52	7-11	258	258	0	105	172	+ 64	1.9
53	10-14	497	497	0	123	181	+ 47	1.5
54	1-7	491	449	— 9	112	123	+ 10	—
55	12-21	510	569	+12	249	542	+118	14.1
56	7-16	346	441	+27	142	288	+103	11.0
57	23-30	331	238	—28	179	155	— 13	1.0
58	13-38	333	391	+17	153	321	+110	2.1
59	1-15	452	506	+12	206	240	+ 17	—
60	17-28	479	462	— 4	318	483	+ 52	—
61	1-11	388	364	— 6	183	279	+ 52	2.3
62	1-9	316	336	+ 6	188	231	+ 23	—
63	9-19	479	460	— 4	237	185	— 22	3.0

TABLE 3

Insulin sensitivity
Effect of intravenous injection of 0.5 unit of insulin on blood sugar

Rabbit	Dose of G.H. on day of test (mg.)	Day of treatment when tested	Before treatment with G.H. Blood sugar (mg. per cent) Before insulin	Before treatment with G.H. Blood sugar (mg. per cent) Maximum fall after insulin	During treatment with G.H. Before glycosuria Blood sugar (mg. per cent) Before insulin	During treatment with G.H. Before glycosuria Blood sugar (mg. per cent) Maximum fall after insulin	During treatment with G.H. During glycosuria Blood sugar (mg. per cent) Before insulin	During treatment with G.H. During glycosuria Blood sugar (mg. per cent) Maximum fall after insulin	After glycosuria Blood sugar (mg. per cent) Before insulin	After glycosuria Blood sugar (mg. per cent) Maximum fall after insulin	After treatment with G.H. Blood sugar (mg. per cent) Before insulin	After treatment with G.H. Blood sugar (mg. per cent) Maximum fall after insulin
57	30	30	111	53			108	15			104	43
58	4	32,62	112	36			98	11	99	32	97	42
59	5	24,55	140	49	124	23	No glycosuria		117	16	149	62
60	10,20,25	8,33,36	133	37	129 114	19 20	120	15			117	52
61	15	10	150	28			158	7			133	39
62	15	4,19	134	55	142	30	No glycosuria		130	31	113	39
63	15,20	15,28	114	36			138	11	134	31	126	41
Mean of maximum falls in blood sugar (mg. per cent)				42		24		12		28		45

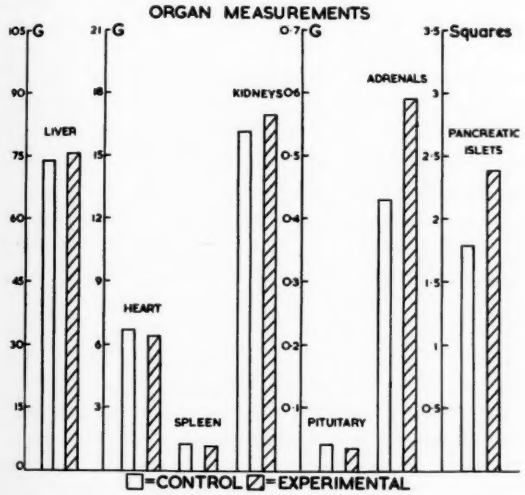


FIG. 5. Organ measurements.

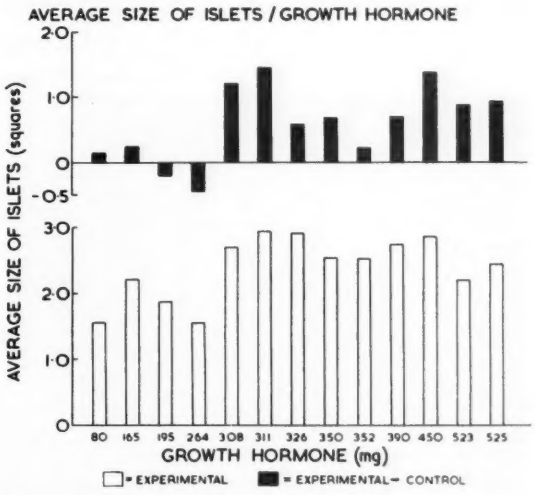


FIG. 6. Average size of islets/growth hormone.

liver, spleen, kidneys, pituitary and adrenals and the mean size of the pancreatic islets in the experimental and control series of rabbits are shown in figure 5. The difference in the mean weights of the heart, liver, spleen, kidneys and pituitary in the two groups was not significant, but the weight of the adrenals and mean size of the pancreatic islets were significantly greater in the experimental than in the control series.

(a) *Pancreatic islets.* The mean size of the islets in the injected rabbits ranged from 1.56 to 2.94 squares (mean 2.39 squares) and was greater than in the paired controls, 1.32—2.32 squares (mean 1.78 squares), in all except two cases (figure 6). The total mean size of the islets was 34 per cent greater in the experimental than in the control animals ($P<1$ per cent). Similarly, the number of large islets (eight squares and over) in the injected group varied, in a count of 225 islets, from three to twenty-three (mean thirteen) and was greater than in the paired controls, two to fourteen (mean seven), except in four cases (figure 7). The total mean number of large islets was 86 per cent greater in the experimental than in the control series ($P<2$ per cent). The increase in islet size and number of large islets in the injected animals was in both instances directly related to the amount of growth hormone administered ($P<5$ per cent), but showed no significant relationship to any glycosuria or increase in body weight. The mean percentages of α and β cells—21.1 ± 5.0 and 78.9 ± 5.0 respectively—in the experimental group were not significantly different from those in the control series—24.3 ± 8.4 and 75.7 ± 8.4 respectively (table 4).

(b) *Adrenals.* The weight of the adrenals in the

injected animals ranged from 0.30 gm. to 0.84 gm. (mean 0.59 gm.) and was greater than in the paired controls, 0.34—0.60 gm. (mean 0.43 gm.), in all except three cases (figure 8). The total mean weight of the adrenals was 37 per cent greater in the experimental than in the control group ($P<5$ per cent). In the injected series, however, no significant relation existed between the increase in weight of the adrenals and the amount of administered growth hormone or increase in mean size of the islets or increase in body weight.

Histology. In one of the rabbits (no. 58) there were certain other changes of interest in the pancreas and adrenals.

(a) *Pancreas.* The cells lining the intralobular and small interlobular ducts were often enlarged and occasionally attained very tall, slender proportions. Their nuclei were then enlarged, while their cytoplasm was unusually acidophilic. These changes affected the epithelium in part or all of the circumference of the ducts and, when marked, had sometimes led to almost complete disappearance of the lumen. They were found most often in single ducts, but also involved groups of small ducts such as occasionally occur normally between the lobules (figure 9). Further, intralobular ducts with the characteristic changes often branched so as to replace the usually single intralobular channel by a series of up to half-a-dozen or more ducts. The altered single ducts also showed apposed islets much more often than is usual in the rabbit pancreas (figure 10). These islets were ordinarily attached to only part of the circumference of the ducts, but sometimes encircled the ducts completely. Two islets were occasionally ap-

TABLE 4

Proportion of α and β cells in pancreatic islets

Experimental			Control		
	Percentage			Percentage	
Rabbit	α cells	β cells	Rabbit	α cells	β cells
51	25	75	16	29	71
52	29	71	13	14	86
53	21	79	11	17	83
54	14	86	15	21	79
55	15	85	20	20	80
56	13	89	18	29	71
57	25	75	27	38	62
58	20	80	17	19	81
59	24	76	21	26	74
60	22	78	19	29	71
61	17	83	24	14	86
62	23	77	12	20	80
63	26	74	26	40	60
Mean	21.1±5.0	78.9±5.0	Mean	24.3±8.4	75.7±8.4

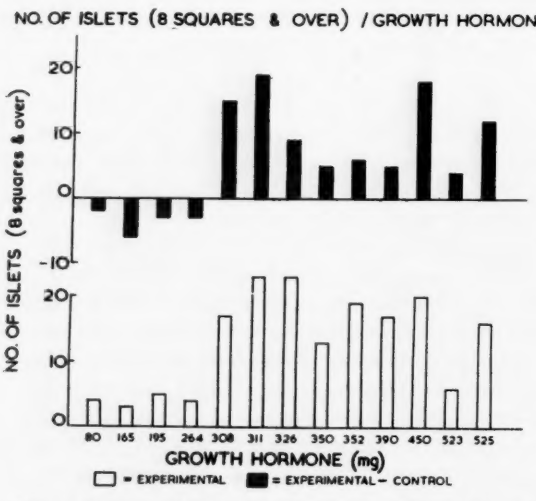


FIG. 7. Number of islets (eight squares and over)/growth hormone.

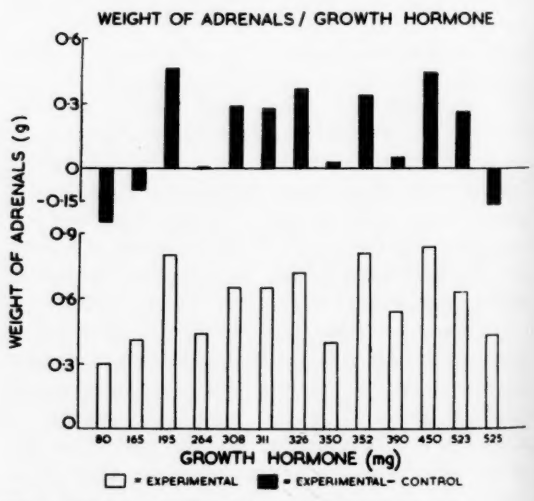


FIG. 8. Weight of adrenals/growth hormone.

posed to the periphery of the same duct. Again, the groups of ducts produced by local branching nearly always exhibited a multifocal apposition of islets, with resultant appearances of very varied character as regards the proportion and relation of the ducts and islets (figure 11). Thus, ducts were sometimes more in evidence than islets and vice versa; or two or three closely approximated ducts enclosed a mass of islet tissue; or ducts and islets were found inextricably mixed. The islets attached to ducts graded in size from those composed of two or three cells to structures at least as large as the average islet and were on the average made up of α and β cells in normal proportions. A clear line of demarcation was always present between the cells lining the ducts and those of the apposed islets, while no mitotic activity was ever detected in the duct or islet cells.

(b) *Adrenals.* One adrenal showed a conspicuous, sharply defined band of hyaline fibrous tissue in the zona reticularis (figures 12 and 13). This band was permeated and covered on both sides by very numerous grossly fatty cells, while many of these cells, especially in the fibrous substance, were in varying degrees atrophied and disintegrated. The other adrenal exhibited more widespread cortical damage (figures 14 and 15). Thus, the zona reticularis was overtaken by a mixture of fatty degeneration, necrobiosis, fibrosis and calcification and included a number of giant, multinucleated, cortical cells. At the same time, the inner part of the zona fasciculata showed a tendency to pyknosis of its nuclei, while the outer segment of this region and usually also the zona glomerulosa were characterized by a frequently

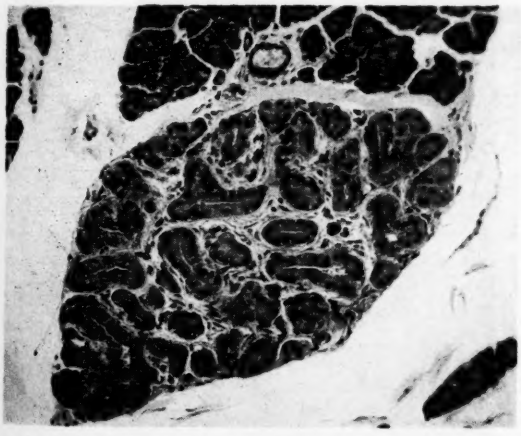


FIG. 9. Rabbit 58. Pancreas. All the ducts in this intralobular group are lined by abnormally tall, acidophile epithelium and characterized by correspondingly reduced lumina. H.E. x 165.

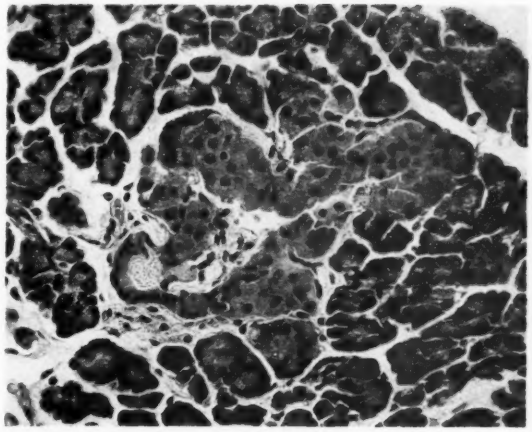


FIG. 10. Rabbit 58. Pancreas. An islet is apposed to an intralobular duct with unusually tall lining. H.E. x 250.

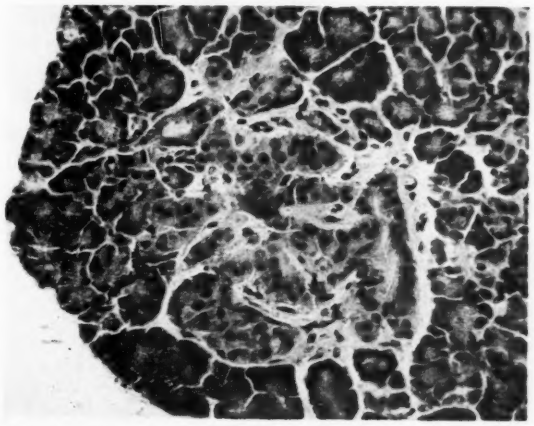


FIG. 11. Rabbit 58. Pancreas. Two intralobular ducts are separated by several small islets, while the ducts are lined by unusually tall, acidophile epithelium. H.E. x 270.

shrunken, hyaline, red- or yellow-stained and even frankly necrotic condition of their cells. The necrotic parts included occasional small foci of polymorphic infiltration.

DISCUSSION

The glycosuria observed in nine of the thirteen rabbits treated with growth hormone corroborates reports of the diabetogenic action of the hormone in the dog,[12] cat,[16,17] rabbit[18] and guinea pig.[18] The dog and cat respond to small doses with a heavy glycosuria whereas in the guinea pig large amounts produce only a trace of glycosuria. Permanent diabetes may even be induced in the dog by relatively small doses.[19] In the present investigation large amounts of the growth hormone caused only a moderate temporary glycosuria, while the individual rabbits also varied considerably in their gly-



FIG. 12. Rabbit 58. Adrenal 1. The zona reticularis shows gross fatty degeneration and fibrosis. H.E. x 7.

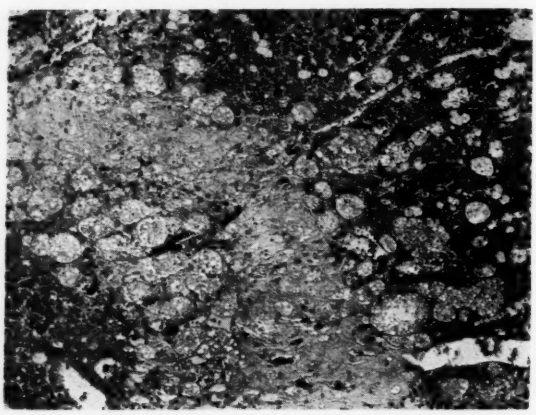


FIG. 13. Rabbit 58. Adrenal 1. Marked fatty degeneration and fibrosis are evident in the zona reticularis. H.E. x 60.

cosuric response to closely similar daily doses of the hormone. Susceptibility to the diabetogenic action of the hormone accordingly depends upon both species and individual. Such species variation is also manifest in the responses of the dog and rabbit to the diabetogenic effects of crude APE. Thus, the dog may be made permanently diabetic by suitable treatment with crude APE[20] whereas corresponding therapy produces only temporary diabetes in the rabbit.[10] This similarity in the



FIG. 14. Rabbit 58. Adrenal 2. The zona fasciculata is degenerated, while the zona reticularis is fatty, fibrosed and calcified. H.E. x 7.

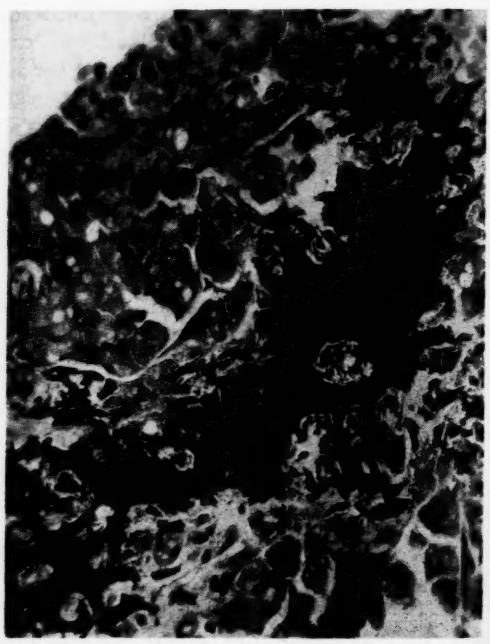


FIG. 15. Rabbit 58. Adrenal 2. This part of the zona reticularis is characterized by a largely calcified, fibrous area and some adjoining fatty degeneration. H.E. x 300.

reactions of the dog and rabbit to both crude APE and growth hormone is in accord with the view that the diabetogenic properties of crude APE may be largely due to its content of growth hormone.[15,16]

The increase in the mean size of the islets and number of large islets induced by growth hormone in the pancreases of these rabbits confirms previous reports of the hyperplastic influence of the hormone on the islets in normal[5,21] hypophysectomized,[5,21] and partially depancreatized[22,23] rats and in pituitary dwarf mice.[24] The changes in the ductal epithelium, proliferation of the small ducts and formation of new islets in the pancreas of rabbit 58 are reminiscent of similar findings in one of a series of rabbits injected with crude APE[10] and recall the genesis of the islets from the small pancreatic ducts during fetal and early postnatal life. The normal proportion of α and β cells found in both the original and new islets of the present injected rabbits also implies an equally marked proliferative influence of the hormone on the two principle types of islet cell. Since the hypophysis is not essential to the maintenance of the islets,[1] the increase in the ducts and islet tissue induced by growth hormone would also appear to be effected indirectly. In this connection some importance may be attached to the reduced sensitivity of these rabbits to the hypoglycemic action of injected insulin during treatment with the hormone, especially in the glycosuric periods. Such a phenomenon has already been observed in the normal rat[25] and hypophysectomized dog[26] and is reminiscent of the same condition in rabbits treated with crude APE.[10,27] Further, a reduction in sugar tolerance follows injections of growth hormone in the normal rat[25] and hypophysectomized dog,[26] while a marked depression occurs in glucose uptake by the excised diaphragm of the hypophyectomized rat after treatment with growth hormone.[28] An inhibition by the hormone of the peripheral utilization of glucose is inferred from these observations and may be regarded as the stimulus to the increase in islet size noted in this investigation. At the same time, no relation was here noted between the amounts of glycosuria and islet hypertrophy so that hyperglycemia, if at all responsible, does not appear to have been the sole stimulus to the islet growth.

The enlargement of the islets induced by crude APE entails an equivalent increase in the amount of extractable insulin.[29] A similar increase in insulin presumably accompanies the islet enlargement resulting from treatment with growth hormone and may well explain the temporary nature of the glycosuria noted in these experiments. The occurrence of only slight glycosuria terminally when the hormone was gradually increased from low to high dosage is particularly suggestive of the progressive acquisition of resistance to the diabetogenic action of the hormone through equivalent islet growth (figure 3). Insulin, however, also induces nitrogen retention.[30–32] Accordingly, an adequate production of insulin in response to treatment with the hormone would not only result in disappearance of any glycosuria, but through nitrogen retention would also in due course initiate an increase in body weight. The inverse relationship between diabetes and growth observed in these experiments is thus attributable to the preponderance of the diabetogenic and pancreotropic effects of the hormone relative to these phenomena. The variation in glycosuric response of species and individual animal to the hormone, as noted earlier, may also be explained on the basis of inverse susceptibility to the pancreotropic action of the hormone. Again, the decrease in nitrogen retention and body weight on an increasing food intake noted immediately after withdrawal of the hormone suggests that the hormone, in the course of bringing about an increase in the amount of islet tissue and extractable insulin, also increases the rate of secretion of insulin. This property has already been inferred from the fall in blood sugar observed in normal and adrenalectomized rats injected with growth hormone.[25]

The increase in urine volume commonly exhibited by the rabbits during treatment can in most cases be interpreted as an effect of the hormone apart from its influence in increasing the food intake or inducing glycosuria, i.e., as due to an enhancing action of the hormone on renal function. Such a view accords with previous observations. Thus, treatment of the hypophysectomized dog with growth hormone results in an increase in the markedly reduced response to water loads, a rise in the daily water exchange, and an improvement in the much impaired glomerular filtration rate and renal plasma flow.[33,34] Again, hypophysectomy lowers PAH and inulin clearances and PAH Tm in dogs whereas growth hormone raises these values in normal dogs to twice the normal and the depressed values of hypophysectomized dogs up to or above the normal.[35–37]

Growth hormone increases the adrenal weight[38,39] and amount of mitotic activity in the adrenal cortex[40–42] of the hypophysectomized rat, while hyperplastic changes may be observed in both cortex and medulla of the adrenals in the intact rat chronically injected with growth hormone.[43] These effects imply adrenotropic activity on the part of the growth hormone and this view is supported by the increase in weight of the adrenals in the present investigation. Such an action, of course,

suggests the possibility that the diabetogenic and pancreotropic effects of growth hormone may be mediated through its action on the adrenals. Insulin resistance, however, can only be induced in rats by combined pituitary and adrenal activity.[4] Moreover, the absence of a significant relationship between the increased weight of the adrenals and the increased size of the pancreatic islets in the rabbits treated with growth hormone makes it unlikely that the islet enlargement noted was mediated wholly through the adrenals.

The degenerative changes in the adrenal cortices of rabbit 58 contrast with the otherwise hyperplastic condition of the adrenals in the treated animals. Rabbit 58 exhibited glycosuria and marked polyuria on a dose as small as 4 mg. hormone daily and was also the only animal to show proliferation of the small pancreatic ducts and a formation of new islets. It was thus exceptionally sensitive to the four actions of the growth hormone—diabetogenic, renotropic, pancreotropic and adrenotropic. The fatty, fibrotic and other degenerative changes noted in the cortices of this rabbit were associated with marked hyperplasia of the glands and so may be regarded as due to exhaustion from overstimulation. Finally, the simultaneous exhibition of glycosuria and adrenocortical damage, in response to treatment with growth hormone, recalls those rare instances of associated diabetes mellitus and Addison's disease in man.

SUMMARY

Growth hormone was given in constant or increasing amounts to thirteen English rabbits for between fifteen and seventy-two days, with the following results:

1. a variable effect upon the appetite of the animals.
2. glycosuria in nine rabbits.
3. an increase in nitrogen retention and body weight in the absence of glycosuria and converse effects during the glycosuric periods and after withdrawal of hormone.
4. an increased output of urine in most animals usually attributable, at least in part, to a direct effect of the growth hormone on the kidney.
5. an increased resistance to hypoglycemic action of injected insulin, particularly during glycosuria.
6. (a) an increase in the mean size of the islets and number of large islets in the pancreases of the treated rabbits, proportional to the amount of hormone administered; (b) a proliferation of the small ducts and a formation of new islets in the pancreas of one treated animal; and (c) no change in the proportion of α and β cells in the pancreatic islets of the treated rabbits.
7. (a) an increase in weight of the adrenals of the treated animals; and (b) striking degenerative changes in the adrenal cortices of one rabbit.

SUMMARIO IN INTERLINGUA

Effecto de Hormon de Crescentia super le Pancreas e le Adrenales de Conilios

Hormon de crescentia esseva administrate in doses constante o in doses crescente a dece-tres conilios anglese durante periodos de inter dece-cinque e septanta-duo dies. Le resultatos esseva le sequentes:

1. Un effecto variabile super le appetito del animales.
2. Glycosuria in novem casos.
3. Un augmento del retention de nitrogeno e del peso corporee in le absentia de glycosuria e effectos converse durante le periodos de glycosuria e post le privation del hormon.
4. Un augmentate production de urina in le majoritate del animales, usualmente attribuibile—al minus in parte—a un effecto directe del hormon de crescentia super le renes.
5. Un augmentate resistentia al action hypoglycemic de injectiones de insulina, specialmente durante le periodos glycosuric.
6. In le pancreas (a) un augmento del dimensiones medie del insulas e un augmento del numero de grande insulas, proportionalmente al quantitate de hormon administrate; (b) un proliferation del micre ductos e le formation de nove insulas in un caso; e (c) nulle alteration in le proportion de cellulas alpha a cellulas beta.
7. In le corpore suprarenal (a) un augmento del peso e (b) frappante alterationes degeneratori del cortice in un caso.

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The Skin-surface-glucose Test

An Aid in the Diagnosis of Diabetes Mellitus

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The present investigation was initiated when diabetic patients using glucose oxidase test paper* for the detection of glucose in the urine reported positive reactions on strips held between the fingers. Although a positive skin-surface-glucose test occurred more frequently when patients showed a positive test for glucose in the urine, at times the skin tests were positive in the presence of negative urine sugar tests. This was evidenced by a color change on the part of the tape held between the fingers while the part dipped into the specimen showed no color change. Contamination was suspected at first, but this possibility was eliminated when positive reactions were observed following washing of the hands prior to testing.

Other investigators have studied the relationship between blood sugar levels and sugar in the skin. Trimble and Carey[1] extracted sugar from human skin in 1932, and found higher values when the blood sugar was elevated. The same investigators reported that the skin of diabetics contained more sugar than the skin of nondiabetics. In 1940 Cornbleet,[2] using dogs, showed an increase in the sugar content of the skin following intravenous dextrose, and a decrease in the sugar content of the skin after intravenous and subcutaneous insulin. Urbach[3] reported a parallelism between skin and blood sugar tolerance curves with one difference—the skin sugar reaches its maximum concentration later and correspondingly takes longer to return to its original level.

Initially, diabetic patients were studied to see if there was any correlation between glycosuria and hyperglycemia with a positive skin test for glucose. The study was then expanded to include nondiabetic patients.

METHODS

Part I

A. *Subjects.* Tests were conducted on eighty-six diabetic and sixty-seven nondiabetic patients of whom forty-eight were male and 105 female. All of the patients were ambulatory. The average weight of the nondiabetics was 157 lb., and the average weight of the diabetics was 168 lb. Patients ranged in age from seventeen to eighty-seven, the median age being fifty-eight years.

B. *Tests.* Tests for glucose on the skin, in urine, and in venous blood were performed on each patient within a period of several minutes. The skin was always tested first to prevent the possibility of contamination during voiding.

1. *Skin.* All possible precautions against contamination of the skin with glucose were observed in performing the skin test. If a patient had eaten prior to the test, he washed his hands with water, and a time interval ranging from thirty to 180 minutes was allowed to elapse before conducting the test. One and one-half-inch strips of glucose oxidase test paper were moistened with tap water. These were held by the patient between the thumb and forefinger of each hand for a period of one minute. Skin tests were recorded as negative if the tape did not change color and positive if a color change occurred.

2. *Urine.* Urine sugars were determined with glucose oxidase test paper and/or copper reagent tablets.* The results were reported either as negative or positive.

3. *Blood Sugars and Tolerance Curves.* True blood sugar levels were determined by the Somogyi-Nelson method[4] using venous blood. A fasting blood sugar level above 120 mg. per 100 ml. or a postprandial blood sugar level above 160 mg. per 100 ml. was considered abnormal.[5] The glucose tolerance test was performed by giving 100 gm. of glucose orally and determining the blood sugars at zero, one half, one, two, and three hours after ingestion of the glucose. Each glucose tolerance test was preceded for three days by a diet containing at least 300 gm. carbohydrate per day (primed glucose tolerance test). Glucose tolerance values above 160 mg. per 100 ml. at the end of one hour and above 120 mg. per 100 ml. at the end of two hours were considered indicative of diabetes mellitus. If the two-hour value was between 110 mg. per 100 ml. and

*Tes-Tape—Eli Lilly and Company.

*Clinitest—Ames Company, Inc.

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120 mg. per 100 ml., the patient was considered a probable diabetic and he was given the cortisone-glucose tolerance test of Fajans and Conn.[6]

The skin and urine were examined prior to starting the glucose tolerance tests and just before drawing each scheduled blood sugar (zero, one half, one, two, and three hours). The hands were washed each time after the skin tested positive.

Part II—Hospital Ward Studies

Four diabetic and two nondiabetic patients were admitted to a hospital ward for the purpose of making more detailed and controlled studies. All patients were ambulatory. The patients were regulated on diet, diet and insulin, or diet and an oral hypoglycemic agent. Food was consumed only at mealtime. Skin, urine, and blood sugar level determinations were performed according to the sample protocol, figure 1. The two nondiabetic controls were tested on three consecutive days in the same manner, figure 1. The four diabetic patients, D.B., C.C., A.M., and J.R., were tested for fourteen, twenty-three, thirty-two, and thirty-four consecutive days, respectively.

RESULTS

Diabetic Patients

The skin test, urine sugar, and blood sugars were obtained on eighty-six known diabetics, table 1. A study of the table shows that seven different combinations of results occurred (Groups I to VII) in the diabetic patients. It was of interest to see that the possible combination of a positive urine, negative skin, and normal blood sugar did not occur in this series.

Elevated blood sugars ranged from 120 mg. per ml. fasting to 565 mg. per 100 ml. postprandial. The degree of hyperglycemia and the amount of glucose on the skin as indicated by the glucose oxidase test paper frequently did not correlate.

Cerebral Vascular Accidents (Group VIII)

Three patients with diagnoses of cerebral vascular accidents and no history of diabetes were found to have positive skin tests, positive urine tests, and elevated blood glucose levels. Their blood glucose levels at the time of the screening tests were 234 mg. per 100 ml., 408 mg. per 100 ml., and 800 mg. per 100 ml.

Nondiabetic Patients (Groups IX and X)

Sixty-four patients were screened by skin, urine, and blood glucose studies, table 1. All sixty-four patients were originally thought to be nondiabetic. Fifty-three of these patients had negative skin, negative urine, and normal blood glucose levels. (Eight of the fifty-three patients were selected at random and given a primed glucose tolerance test—normal glucose tolerance curves resulted.)

The remaining eleven patients had positive skin, negative urine, and normal blood glucose levels. A primed glucose tolerance test was also given to these patients.

PROTOCOL

Patient ..... Hosp. No. .....

Date	Right hand	Left hand	Urine	Blood glucose	Remarks
6:30 a.m. Test fingers and wash hands with water.	____	____			
7:00 a.m. Test fingers, urine, and draw blood glucose.	____	____	____	_____	
Breakfast (8:00 a.m.) After breakfast, wash hands with water until fingers check negative.	____	____			
11:00 a.m. Test fingers, urine, and draw blood glucose.	____	____	____	_____	
Lunch (11:30 a.m.) After lunch, wash hands with water until fingers check negative.	____	____			
4:00 p.m. Test fingers, urine, and draw blood glucose.	____	____	____	_____	
Supper (5:00 p.m.) After supper, wash hands with water until fingers check negative.	____	____			
10:00 p.m. Test fingers, urine, and draw blood glucose.	____	____	____	_____	

DON'T USE SOAP — AIR DRY

FIG. 1. Sample protocol for determining skin, urine, and blood glucose levels on hospitalized patients.

TABLE 1

Summary of initial screening tests on 153 patients

Groups	Number of patients	Clinical diagnosis	Tests for glucose Skin	Urine	Blood
I	19	Diabetes mellitus	—	—	—
II	42	Diabetes mellitus	+	+	+
III	2	Diabetes mellitus	—	+	+
IV	5	Diabetes mellitus	—	—	+
V	7	Diabetes mellitus	+	—	+
VI	3	Diabetes mellitus	+	+	—
VII	8	Diabetes mellitus	+	—	—
VIII	3	"CVA"	+	+	+
IX	53	Controls	—	—	—
X	11*	"Controls"	+	—	—

*Two patients had normal glucose tolerance curves. Two other patients had glucose tolerance curves which were borderline normals; cortisone-glucose tolerance tests on these two patients were abnormal. Seven of these patients had glucose tolerance curves indicative of diabetes.

In this series the possible combination of a positive urine, negative skin-surface-glucose test, and normal blood level of glucose was not observed.

Only two of these patients had normal curves; two other patients had a two-hour value between 110 mg. per 100 ml. and 120 mg. per 100 ml. Since these values were borderline, cortisone-glucose tolerance tests were made. Abnormal curves were obtained in each instance, figure 2.

Seven patients who had positive skin tests but negative urines and normal blood glucose levels during the initial testing had glucose tolerance curves indicative of diabetes, figure 3. Repeated glucose tolerance tests in two patients confirmed the initial abnormal findings. All seven patients had one-hour blood glucose levels above 160 mg. per 100 ml. and two-hour levels about 120 mg. per 100 ml. At the end of three hours, four of the seven patients had blood glucose levels above 140 mg. per 100 ml.

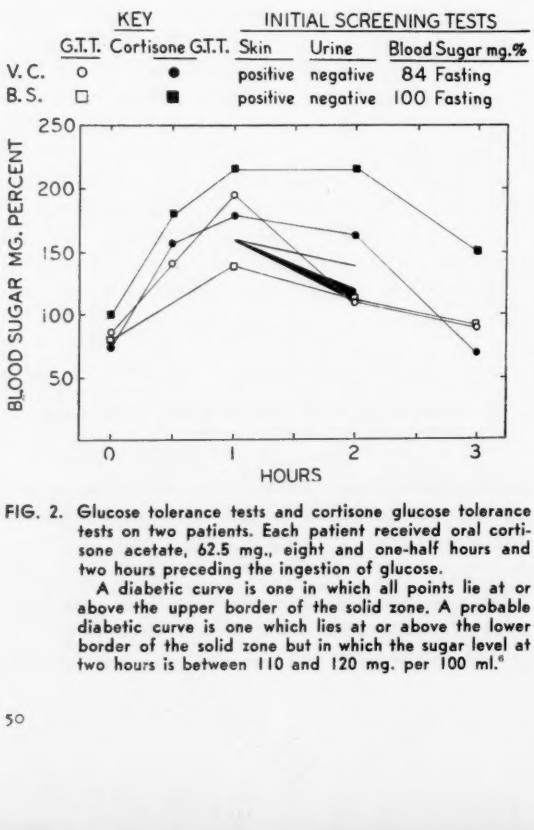


FIG. 2. Glucose tolerance tests and cortisone glucose tolerance tests on two patients. Each patient received oral cortisone acetate, 62.5 mg., eight and one-half hours and two hours preceding the ingestion of glucose.

A diabetic curve is one in which all points lie at or above the upper border of the solid zone. A probable diabetic curve is one which lies at or above the lower border of the solid zone but in which the sugar level at two hours is between 110 and 120 mg. per 100 ml.[6]

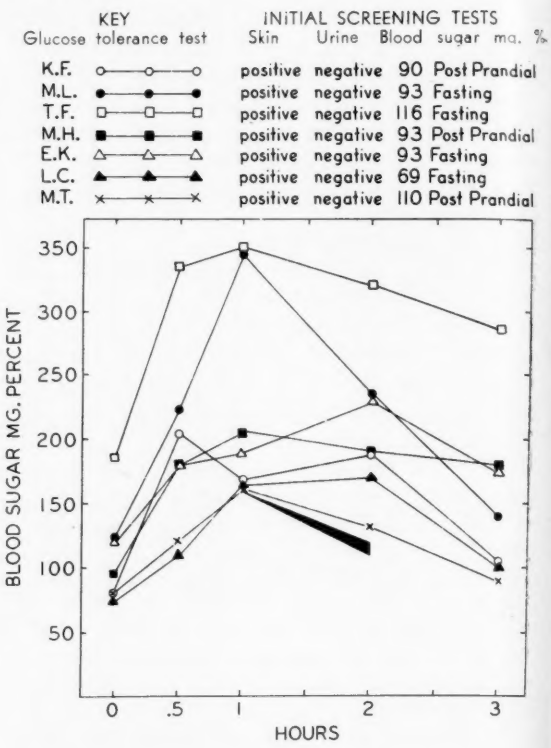


FIG. 3. Glucose tolerance tests on seven patients who on initial screening tests showed positive skin, negative urine, and normal blood glucose levels.

All one-hour determinations above 160 mg. per 100 ml. and two-hour determinations above 120 per 100 ml.

Skin, Urine, and Glucose Tolerance Curves

Table 2 demonstrates results of skin, urine, and blood glucose levels during glucose tolerance tests on a known diabetic, a probable diabetic, and a nondiabetic patient.

S.W., a known diabetic, with an abnormal glucose tolerance test, did not show any glycosuria during the entire three hours of the glucose tolerance test; however, it was noted that the skin tests were positive at one-half, two, and three-hour time intervals in the left

TABLE 2

Skin, urine, and glucose tolerance curves

Time	Hands* Right	Hands* Left	Blood sugar mg. per cent	Urine
S.W.—known diabetic				
Fasting				
100 gm. glucose	Negative	Negative	110	Negative
½ hour	Negative	Positive	180	Negative
1 hour	Negative	Negative	204	Negative
2 hours	Positive	Positive	184	Negative
3 hours	Positive	Positive	156	Negative
V.C.—"probable diabetic"				
Fasting				
100 gm. glucose	Negative	Negative	86	Negative
½ hour	Negative	Positive	142	Negative
1 hour	Negative	Positive	194	Positive
2 hours	Negative	Negative	110	Positive
3 hours	Negative	Positive	90	Negative
H.R.—nondiabetic				
Fasting				
100 gm. glucose	Negative	Negative	86	Negative
½ hour	Negative	Negative	116	Negative
1 hour	Negative	Negative	100	Negative
2 hours	Negative	Negative	84	Negative
3 hours	Negative	Negative	72	Negative

*If skin test was positive, hands were washed with water until test became negative.

hand. A positive skin-surface-glucose test did not appear on the right hand until two hours after the glucose load was taken.

V.C., a probable diabetic, was found to have a normal fasting blood glucose, a negative urine test, but a positive skin test on the initial screening procedure. With a primed glucose tolerance test, glycosuria was positive at the one- and two-hour time intervals and negative after three hours. Although the blood sugar levels of the glucose tolerance test indicated the diagnosis of probable diabetes, positive skin tests occurred on the left hand only at one-half, one, and three hours. A follow-up cortisone-glucose tolerance test was positive.

H.R., a nondiabetic, demonstrated a normal glucose tolerance curve, aglycosuria, and negative skin tests.

Hospitalized Patients

The studies on the hospital patients which permitted frequent testings under closer observation showed a good relationship between the presence of glucose on the skin and hyperglycemia. When A.M. and J.R. were given 2,200-calorie diets, the skin tests were repeatedly positive with corresponding hyperglycemia and glycosuria. A reduction in calories in these two patients caused a lowering of the blood sugar with a decreased number of positive skin tests. No positive tests (skin, urine, or elevated blood glucose levels) were ever found on the control patients.

DISCUSSION

In this study, a difference in the amount of glucose present on the skin of diabetics and nondiabetics appears to be correlated with the blood glucose level. This finding suggests that Urbach's concept that the skin serves as a temporary storehouse for sugar and plays an important role in intermediary carbohydrate metabolism deserves consideration. An association between elevated blood sugar and skin diseases has prompted many clinicians to treat pustular skin conditions with small doses of insulin. More recently, Cohen and Cohen[7] reported twenty-six cases of pustular acne vulgaris and other pustular dermatoses resistant to the usual forms of therapy. When these patients were treated with an oral hypoglycemic agent, twenty-five cases improved. The skin comprises 16 per cent[8] of the total body weight and could be a very important organ in carbohydrate metabolism.

The data presented suggest a correlation between hyperglycemia, glycosuria, and positive skin glucose reactions; however, at times the skin test was negative when hyperglycemia and glycosuria were present.

A more important finding was the presence of a positive skin test in the absence of glycosuria and hyperglycemia. Therefore, this test may permit the detection of abnormal carbohydrate metabolism when other tests appear normal. Urbach's observation[3] that the skin sugar reaches its maximum concentration later than the blood, and takes longer to return to its original level, may explain some discrepancies between the blood sugar level and the skin-surface-glucose test.

Seven newly discovered diabetics and two probable diabetics, table 1, were found while screening sixty-four patients. The only finding originally observed in these nine patients to suggest diabetes was a positive skin-surface-glucose test. Blood glucose levels and urine sugar tests were negative. A glucose tolerance test confirmed the presence of an abnormal glucose metabolism. These results indicate that the skin-surface-glucose test may be useful in conjunction with other technics to reveal undiscovered or prediabetic patients. The procedures which have been used in mass screening programs to date have disclosed only 1 to 2 per cent new diabetics.[9-11]

Approximately 11 per cent new diabetics were revealed by the skin-surface-glucose test.

This preliminary study has shown glucose oxidase test paper to be a useful adjunct for the detection of patients with hyperglycemia. However, several ques-

tions require further investigation. During the glucose tolerance tests, skin-surface-glucose tests were positive within a period ranging from one-half to four hours after the glucose load. An optimal time for the skin-surface-glucose test following the ingestion of food has not been established. A curious finding was the presence of a positive skin-surface-glucose test on the fingers of one hand and a negative reaction on the fingers of the other hand even though the tests were conducted simultaneously.

SUMMARY

A simple skin test using glucose oxidase test paper for the detection of glucose on the skin has been described. Studies were made to define the relationship between glycosuria, hyperglycemia, and the presence of glucose on the skin. In the course of the study a number of new diabetics were discovered. Nine of sixty-four patients in whom diabetes was not suspected had a positive skin test during initial screening and normal blood glucose levels and urine sugar tests. Follow-up glucose tolerance tests were indicative of the presence of diabetes in seven of these patients. The cortisone-glucose tolerance test was needed to confirm the presence of probable diabetes in two others. A positive skin test appears to indicate that the blood glucose is or has recently been elevated. This could be a useful adjunct to present-day screening technics for the detection of diabetes, especially in patients with a high renal threshold and/or normal fasting blood sugars.

SUMMARIO IN INTERLINGUA

Un Test pro Glucosa al Superficie del Pelle: Un Adjuta in le Diagnose de Diabete Mellite

Es describite un simple test cutanee, utilisante papiro reactive a oxydase de glucosa pro le detection de glucosa al superficie del pelle. Esseva interprendite studios pro definir le relation inter glycosuriae hyperglycemia e le presentia de glucosa al superficie del pelle. In le curso del studios, un numero de nove diabeticos esseva discoperite. Novem ex sexanta-quatro patientes in qui nulle diabete habeva essite suspicite habeva un positive test cutanee in le triation initial sed normal nivellos de glucosa sanguinee e normal tests pro sucro sanguinee. Tests consecutori de toleration pro glucosa indicava le presentia de diabete in septe de iste patientes. Le test del tolerantia pro cortisona e glucosa esseva necessari pro confirmar le presentia de diabete probabile in duo alteres. Un positive test cutanee pare indicar que le glucosa del sanguine es currentemente o esseva recentemente elevate. Isto poterea esser utile como adjuncto al technicas currente pro le detection de diabete, specialmente in patientes con un alte limine renal e/o valores normal pro sucro sanguinee in stato jejun.

ACKNOWLEDGMENT

Valuable assistance was received from our colleagues, nurses, and laboratory technicians.

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Studies on the Diabetogenic Action and the Site of Origin of Glucagon

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Although the acute hyperglycemic action of glucagon is an established fact there are relatively few reports as to the effect of its chronic administration on blood sugar and pancreatic morphology. The rat,[1-3,5,6] rabbit,[3] cat[4] and dog[4-6] have, in general, been reported to be relatively insensitive to its hyperglycemic action. However, in one study large amounts of glucagon given to rats for a period of ten days have been shown to produce a diabetic effect.[5,6] There are only two reports which we know of by other workers as to the effect of glucagon on pancreatic morphology. Thus, Kracht[7,8] noted involution of the pancreatic α cells and degranulation and hypertrophy of the β cells after prolonged glucagon administration in the rabbit and rat. Salter and his associates[6] reported transient hyperglycemia and mild glycosuria in two dogs after seven days of glucagon administration, with either more intense granulation or degranulation and hydropic degeneration of the β cells as well as marked atrophy of the acinar cells. However, no α cell changes were mentioned.

The present study is a summary of our work concerning the effect of prolonged glucagon administration on the pancreas and on blood and/or urinary sugar in rabbits,[9] guinea pigs[10] and dogs. Furthermore, in the dog, in order to determine whether a severe degree of diabetes could be produced, the insulinogenic reserve was reduced by partial pancreatectomy. The rabbits and dogs received subdiabetogenic dosages of cortisone simultaneously with the glucagon since this hormone has previously[11,12] been observed by us to potentiate the hyperglycemic action of glucagon.

MATERIAL AND METHODS

1. *Rabbits.* Forty-six New Zealand white rabbits of either sex, weighing between 2.5 and 4 kg., were divided into four groups: Group 1 consisted of twelve untreated control rabbits. Group 2 comprised ten rabbits who each received subcutaneously 1 mg. of crystalline glucagon (Lilly)* suspended in corn oil three times daily, at 10 a.m., 4:30 p.m. and 12 midnight. Six animals were sacrificed between the forty-ninth and seventy-eighth days. The remaining four rabbits expired between the sixty-fifth and eightieth days. Group 3 consisted of eighteen rabbits who each were given 1 mg. of crystalline glucagon subcutaneously daily at intervals as in Group 2 together with 1 mg./kg. daily intramuscularly of cortisone acetate. Eleven rabbits were sacrificed between the fifth and 104th days. Seven animals expired at intervals from four to thirty days after the start of treatment. Group 4 consisted of six rabbits each of which received 1 mg./kg. intramuscularly of cortisone acetate daily and were sacrificed at eighteen to seventy-two days.

2. *Guinea pigs.* Thirty animals of either sex, weighing between 700 and 1,000 gm., were divided into three groups: Group 1 consisted of twelve guinea pigs each of which received 0.33 mg. of glucagon subcutaneously suspended in corn oil three times daily, at 9:30 a.m., 4:30 p.m. and 12 midnight. Six of these animals expired. The other six were sacrificed at twenty-eight to 124 days. Group 2 consisted of six guinea pigs, each of which received subcutaneously 30 mg./kg. of cobaltous chloride as 1 per cent solution for four consecutive days and were sacrificed on the morning of the following day. Group 3 consisted of twelve guinea pigs which served as untreated controls.

3. *Dogs.* Six mongrel dogs of either sex, weighing between 12 and 15 kg., were 50 to 80 per cent pancreatectomized. Beginning on the fourteenth postoperative day they each received daily 1 mg./kg. of cortisone acetate in aqueous suspension. In addition, three of these animals received 5 mg. of crystalline glucagon (Lilly) suspended in corn oil subcutaneously twice daily, at 10 a.m. and 4:30 p.m. These dogs were sacrificed at twenty-seven, fifty-two and ninety days after starting

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*Crystalline glucagon (Lot #258-234B-54-2) was supplied by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, through the courtesy of Dr. W. R. Kirtley.

hormone therapy. The remaining three animals that received cortisone alone were sacrificed on the twenty-eighth, forty-ninth and seventieth days.

All animals were kept in individual metabolic cages. The rabbits and guinea pigs received Purina Rabbit Chow, carrots and tap water ad libitum. All dogs received a high carbohydrate diet consisting of mashed potatoes, meat and meat gravy, and 5 per cent glucose solution ad libitum instead of drinking water. Daily twenty-four-hour urine specimens were collected under toluol. Each animal received 200,000 units of procaine penicillin G and 0.25 gm. of dihydrostreptomycin sulfate (Combiotic Pfizer) twice weekly. Blood from the rabbits was drawn from the marginal ear vein daily in the morning at approximately 9:30 a.m. and in the afternoon at approximately 1:30 p.m. Blood from the dogs was drawn once daily from the leg veins. The blood and urinary glucose were determined by the Nelson-Somogyi micromethod.[18]

All surviving animals were sacrificed by overdosage with sodium nembutal and pancreatic tissue of the sacrificed, but not of the animals which expired spontaneously, was placed immediately into Zenker-formol 20 per cent solution for histologic studies. The blocks were embedded in paraffin and sections were stained by the following methods: Iron hematoxylin, phosphotungstic acid hematoxylin, modified Masson trichrome,[14,15] a modification of the aldehyde-fuchsin trichrome,[16] a modification of Gomori's chrom alum hematoxylin[15,17] and the Periodic Acid Schiff technic with a trichrome counterstain[15] controlled by diastase for glycogen identification.

RESULTS

1. *Rabbits. Group 1.* The untreated control rabbits showed reducing substances in the urine which varied from 0 up to 0.9 gm. in twenty-four hours. Therefore, values above 1 gm. per day were considered significant. There were no marked differences between the morning and afternoon blood sugar levels which varied from 93 to 158 mg. per cent. A nonfasting blood sugar of greater than 160 mg. per cent was considered a significant elevation.

In the normal rabbit pancreas the α cells were clearly identifiable by their intensely acidophilic and siderophilic cytoplasm (figure 1). However, not every islet contained these cells. All the small intralobular ductules had a similar histologic appearance and continuity between these structures and the islets of Langerhans was only rarely observed.

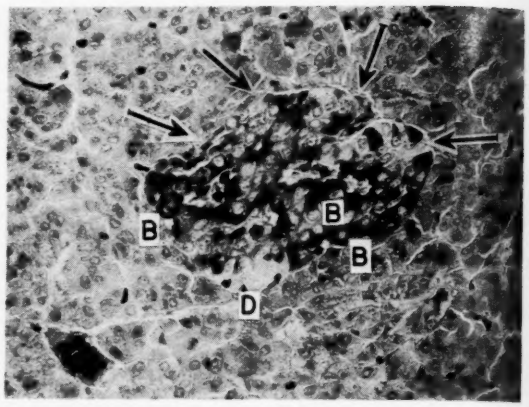


FIG. 1. Islet from pancreas of untreated rabbit showing groups of α cells (arrows) with gray cytoplasm, dark β cells (B) and clear δ cells (D). Aldehyde fuchsin trichrome stain, × 300.

Group 2. The animals treated with glucagon alone showed intermittent hyperglycemia with morning blood sugars varying from 82 to 184 mg. per cent and afternoon levels ranging from 105 to 420 mg. per cent. The total twenty-four-hour urinary glucose excretion varied from 0.2 to 6 gm. In general, the hyperglycemia and glycosuria were more severe in the early phases of the experiments and tended to decline during the course of treatment. The blood and urinary sugar values for a representative rabbit are demonstrated in figure 2A.

In the pancreas there was a decrease of identifiable α cells, while those remaining were frequently partly degranulated. Intrainsular cells with faintly staining cytoplasm and dark, somewhat pyknotic, nuclei were noted which were interpreted as representing completely degranulated α cells. There were also groups of small chromatic nuclei with unstained cytoplasm which were believed to be involuted α cells. They were quite different from the nuclei of adjacent β cells which were much larger, more ovoid and had more sparsely distributed chromatin.

Group 3. The rabbits which were treated simultaneously with glucagon and cortisone displayed a severe but intermittent hyperglycemia. During the early stages the morning blood sugar values were frequently within normal limits. Thereafter, however, they rose as high as 450 mg. per cent. The afternoon blood sugar levels were increased from the very beginning. This elevation became quite marked as treatment continued, with occasional blood sugar values as high as 865 mg. per cent. After six weeks the morning and afternoon glycemic levels were frequently less than prior to that time. The daily

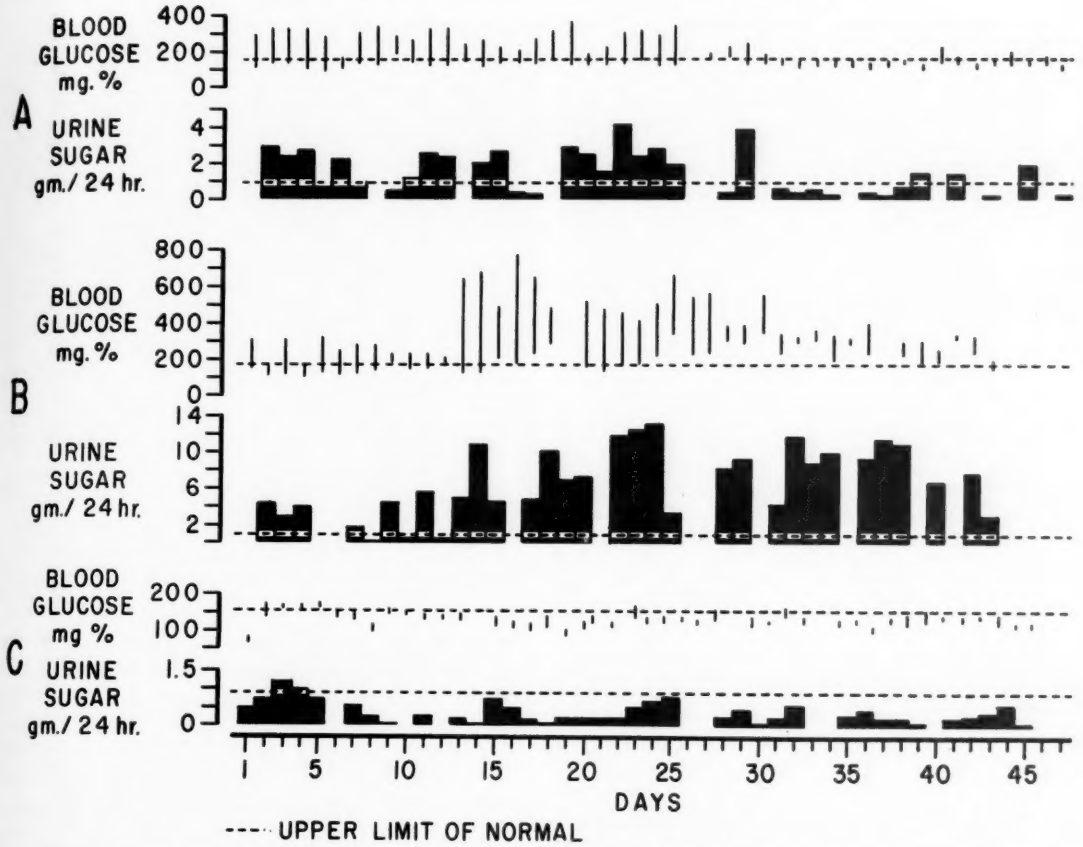


FIG. 2. Daily morning and afternoon blood sugar levels as well as daily twenty-four-hour urinary glucose output of three representative rabbits who received respectively: (A) 1 mg. of crystalline glucagon in corn oil subcutaneously three times daily. (B) Glucagon as in (A) as well as 1 mg./kg. daily of cortisone acetate intramuscularly. (C) Cortisone acetate alone as in (B).

urinary glucose excretion was markedly increased, reaching as much as 12 gm. and, in general, paralleled the blood sugar levels. The daily blood sugar concentrations as well as the twenty-four-hour glucose output of a representative animal are demonstrated in figure 2B.

The α cell changes in this group were similar to those observed in rabbits treated with glucagon alone. Under low power they appeared to be markedly diminished in number (compare figures 3 and 4). Under high power they showed partial degranulation and nuclear pyknosis (figure 5). In addition there were frequently groups of chromatic nuclei with unstained cytoplasm thought to be completely degranulated involuted α cells (figure 6).

The rabbits which received glucagon and cortisone for long periods showed a marked increase in volume of the pancreatic islets (figure 4). The β cells were usually degranulated and increased in size and the nuclei were enlarged. There was hyperplasia of the epithelium of some intercalated ductules which were frequently contiguous with or even within pancreatic islets, and neogenesis of β cells from proliferating ductular epithelium could be traced (figure 7). New β cells originally appeared as large cells with opaque, slightly granular and somewhat reticulated cytoplasm. At an early stage the immature β cells were large and contained sparse, diffuse aldehyde fuchsin positive granules within the cytoplasm. With progressive maturation of these cells, the aldehyde fuchsin positive granules became more abundant throughout the cytoplasm. They could be readily distinguished from older β cells which were smaller, degranulated by hyperglycemia and showed aldehyde fuchsin positive cell borders.

In the severely diabetic rabbits there was marked

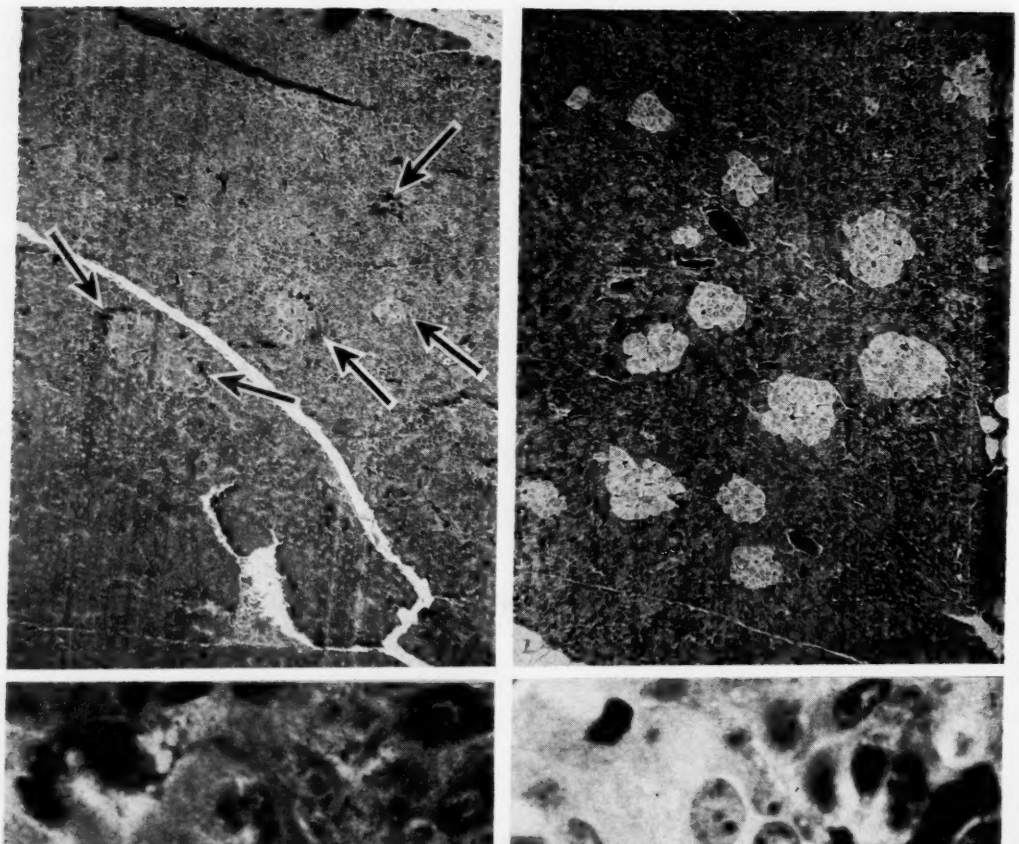

A
A
B
B

FIG. 3. (Upper left, page 56) Pancreas of untreated rabbit showing islets with distinct α cells (arrows). Periodic Acid Schiff trichrome stain, × 145.

FIG. 4. (Upper right, page 56) Pancreas of a rabbit treated with cortisone and glucagon for 106 days, showing marked islet hyperplasia and no apparent α cells. Periodic Acid Schiff trichrome stain, × 188.

FIG. 5. (Lower left, page 56) Pancreatic islet from same rabbit as in figure 3, showing α cells (A) with poorly stained cytoplasm. Their nuclei are somewhat smaller and more chromatic than those of the adjacent β cells (B). Modified Masson trichrome stain, × 1,280.

FIG. 6. (Lower right, page 56) Another pancreatic islet from same rabbit as in figures 3 and 4. There is a group of small chromatic nuclei (arrows) which are surrounded by poorly stained cytoplasm thought to represent degranulated involuted α cells. The nuclei of the adjacent β cells are much larger and less chromatic. Masson trichrome stain, × 1,600.

glycogen infiltration (vacuolization) of the ductular epithelium and of many of the β cells. This glycogenic vacuolization was only observed in some ductules while in the proliferating ductules this material was not seen. Similarly, the cells identified as young β cells did not contain glycogen even while this was present within the other β cells.

Group 4. In the rabbits treated with cortisone there was little difference between the morning and afternoon blood sugar values which varied from 68 to 185 mg. per cent. The twenty-four-hour urinary glucose excretion ranged from 0 to 0.4 gm. Representative blood sugar values and urinary glucose output are presented in figure 2C. In this group there were no changes in the pancreatic α cells. The β cells showed moderate degranulation, very mild degrees of glycogen infiltration and modest ductular and islet hyperplasia. The islet hyperplasia appeared similar in degree to that observed in animals treated for equal periods of time with glucagon and cortisone. However, it was considerably greater than that observed with glucagon alone.

2. *Guinea pigs.* In the normal guinea pigs the output of urinary reducing substances varied between 0 and 109 mg. in twenty-four hours. The animals treated with glucagon had a urinary glucose excretion which ranged from 1.5 mg. to as much as 2.6 gm. within twenty-four hours.

Morphologically, in the normal guinea pigs the α, β and δ cells (figure 8) were readily identified. There were also occasional cells with faintly staining eosinophilic or unstained cytoplasm which were neither α, β nor δ cells. In addition, interacinar acidophilic and siderophilic cells, the so-called x cells[17,19,20] were identified. The cobalt-treated animals showed in varying degrees the typical punched-out lesions of the α cells[21,22] but no other pancreatic changes.

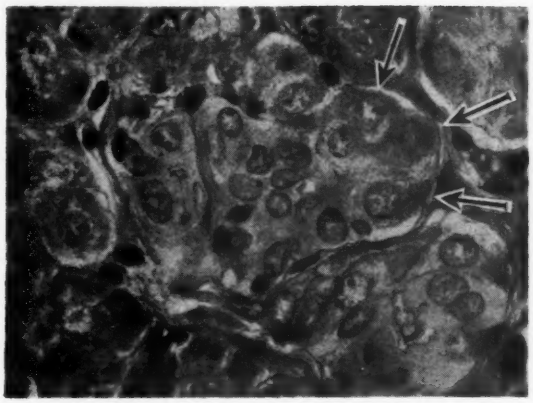


FIG. 7. Pancreas of same rabbit as in figure 4, showing proliferating hyperplastic ductules capped by newly formed granulated β cells (arrows). Aldehyde fuchsin trichrome stain, × 760.

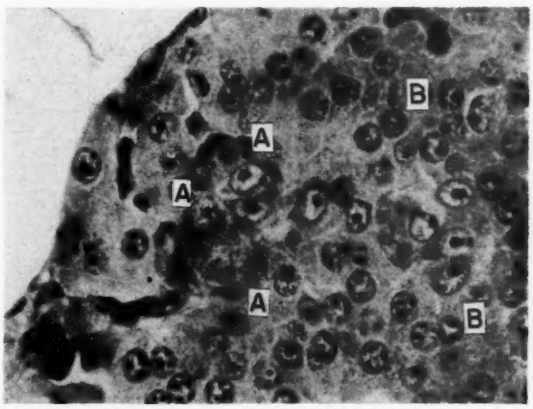


FIG. 8. Pancreatic islet of untreated guinea pig showing α cells (A) with dark granular cytoplasm and pale β cells (B). Periodic Acid Schiff trichrome stain, × 760.

The glucagon-treated guinea pigs which were sacrificed at twenty-eight days showed an apparent loss of α cells. However, on closer inspection intra-islet cells with faintly acidophilic or siderophilic cytoplasms were seen, which were considered to be degranulated α cells. Additional cells were observed with faintly staining cytoplasm which could not be definitely identified as α cells. The β cells were well granulated. At forty-two days, there were a smaller number of clearly identifiable α cells present. Animals treated for ninety-five days showed a marked decrease of stainability of the cytoplasm of the α cells. There was considerable hyperplasia of the islets and very moderate β cell degranulation. These observations were accentuated in animals treated for 124 days in whom only occasional faintly staining

α cells were noticeable. Furthermore, cells with somewhat reticulated foamy and unstained cytoplasm were noted which were considered to be degranulated α cells (figure 9). There was but moderate degranulation of the β cells.

3. *Dogs.* The morning blood sugars and the twenty-four-hour urinary glucose excretion for each of the three dogs treated with glucagon and cortisone are represented in figure 10. It can be seen that dogs A and B developed only a moderate morning hyperglycemia. Dog C, after a period of two weeks, showed an elevated morning blood sugar which eventually rose as high as 400 mg. per cent.

Morphologically, the partially pancreatectomized cortisone treated control dogs showed normal islets (figure 11). The glucagon plus cortisone-treated partially depancreatized animals revealed no definite α cell changes.

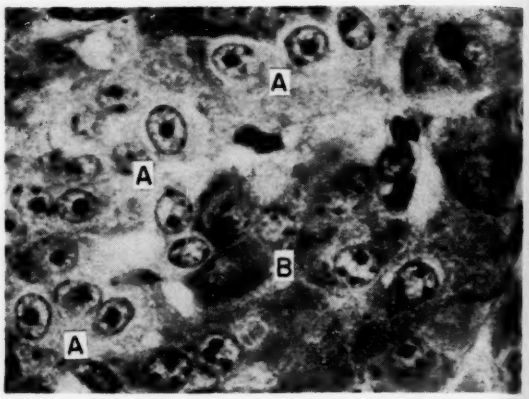


FIG. 9. Pancreatic islet of guinea pig treated with glucagon for 124 days showing degranulated α cells (A) and intact β cells (B). Aldehyde fuchsin stain, × 1,013.

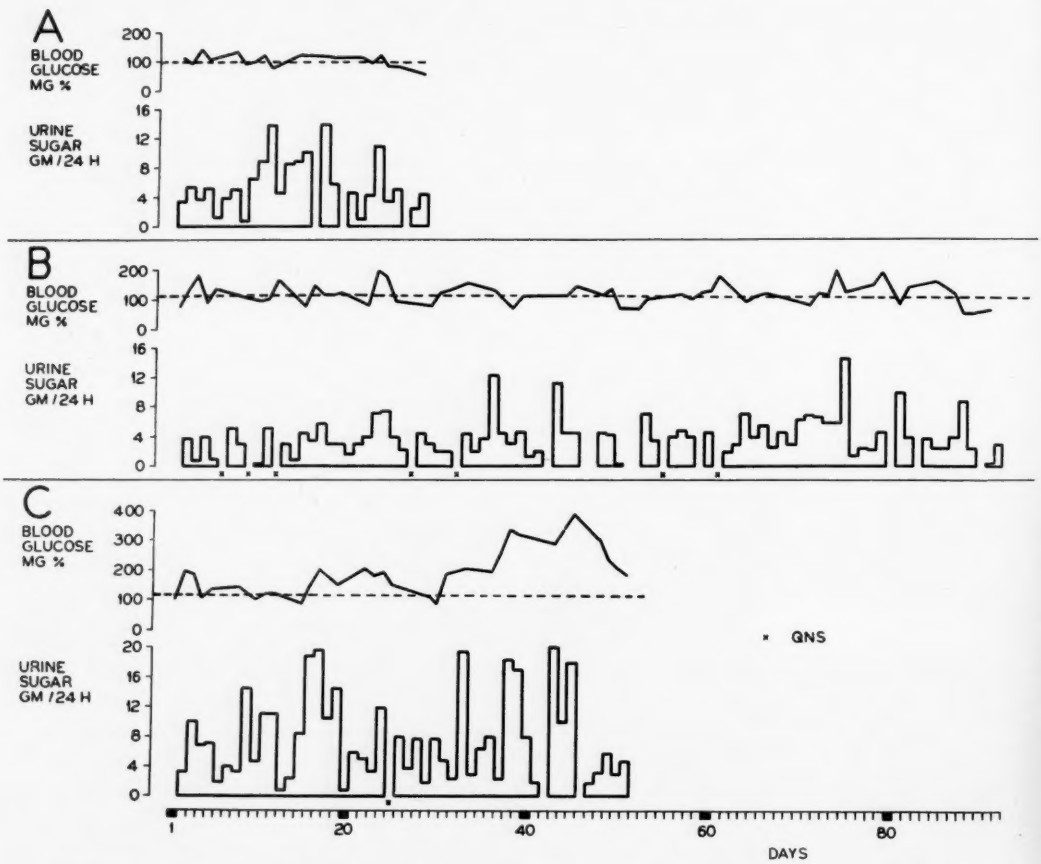


FIG. 10. Daily morning blood sugars of three partially pancreatectomized dogs who received 5 mg. crystalline glucagon in corn oil twice daily subcutaneously as well as 1 mg./kg. daily of cortisone acetate intramuscularly. The interrupted line indicates the mean of the pre-injection morning blood sugar levels.

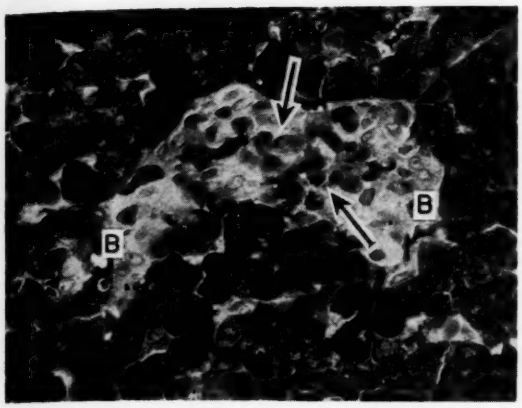


FIG. 11. Islet from pancreas of untreated dog showing the dark α cells (A) with dark granular cytoplasm and pale β cells (B) and δ cells (D). Modified Masson trichrome stain, × 317.

However, other alterations usually associated with hyperglycemia were found. Dog A displayed moderate degranulation of the β cells, as well as a small increase in the amount of Schiff positive material found within pancreatic ductular epithelium, and increased ductular vacuolization. Dog B displayed increased glycogen deposition in pancreatic duct epithelium and glycogen was also occasionally found within β cells, but there was only questionable β cell degranulation. The pancreatic remnants (50 per cent) of these two animals were fairly normal in appearance and showed almost no fibrosis. Dog C, which was 80 per cent pancreatectomized, displayed considerable interlobular pancreatic fibrosis (figure 12). Islet tissue was not immediately apparent on casual inspection but on closer examination residual islets were seen which were composed of α cells, and nongranular cyanophilic cells with clear cytoplasm. Throughout the pancreas there was marked vacuolization of duct epithelium and of centro-acinar cells which contained mostly glycogen (figure 13).

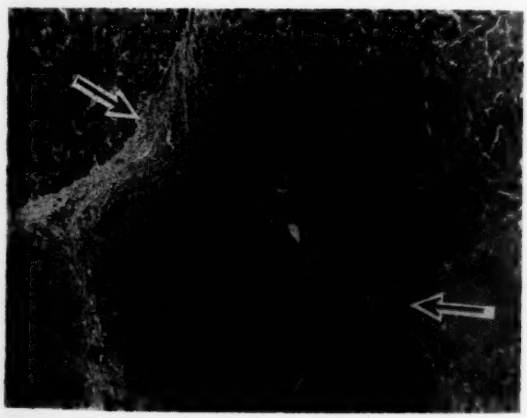


FIG. 12. Low power view of pancreas of Dog C which was 80 per cent partially pancreatectomized and treated with glucagon and cortisone for fifty-two days. There is marked interlobular fibrosis (arrows) and heavy glycogen deposits in ductular epithelium (black). Chrom alum hematoxylin phloxin stain, × 50.

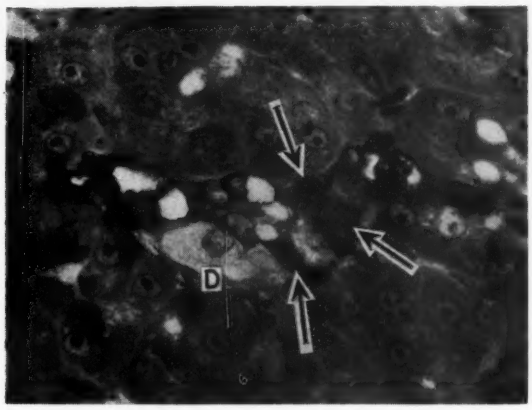


FIG. 13. Islet from pancreas of Dog C showing granulated α cells (arrows), a cyanophilic cell, possibly a δ cell (D) as well as vacuolated ductular epithelium containing glycogen (black). Periodic Acid Schiff trichrome stain, × 760.

DISCUSSION

In accord with the results of others, it has been demonstrated that chronic administration of glucagon alone has only minimal diabetogenic action.[1-6] This finding is at variance with the suggestion that increased glucagon production might be a cause of human diabetes.[23] On the other hand, conjoint administration of glucagon and cortisone in the rabbit resulted in a more severe and prolonged diabetes than ever before produced in this species by hormonal action. This finding is in accord with our previous observation[11,12] that, in the rabbit, cortisone administration potentiates the hyperglycemic effect of glucagon. It is also in agreement with the reports of others[1] that adrenocorticotrophic hormones and cortisone exaggerate glucagon-induced hyperglycemia in the rat.

Morphologically, the most striking observation in the guinea pigs and rabbit was the partial or complete loss of α cell cytoplasmic granulation seen in both species as well as the nuclear pyknosis found in the rabbit. This lesion in guinea pigs is quite different from the punched-out destruction of α cells seen in similar animals treated with cobaltous chloride.[21,22] These results

are in accord with another study in which atrophy of the α cells was found in rabbits and rats after glucagon administration.[7,8] Other workers did not mention pancreatic α cell changes in rats or dogs after several days of treatment with glucagon in corn oil.[5,6] The degranulation of rabbit and guinea pig α cells is considered to represent the suppression of endogenous secretion of a hormone by its exogenous administration. Similar phenomena have been previously described with other hormones. These observations support the viewpoint that glucagon is a hormone produced by the α cells and that it has a physiologic action.[24,26]

The other pancreatic morphologic changes observed are similar to those reported after various types of hormonally induced experimental diabetes.[27,28] These included β cell degranulation, glycogen infiltration of ductular and β cells as well as islet hyperplasia. These morphologic alterations are a resultant of hyperglycemia and are particularly pronounced in the severely diabetic animal.[29–40] Furthermore, it has been shown that, in general, ductular glycogen deposition precedes its appearance in β cells and that this phenomenon is only one aspect of the ubiquitous deposition of glycogen which occurs during hyperglycemia.[35–40]

The present experiments again demonstrate the previously reported phenomenon[41–47] of adaptation to hormonally induced hyperglycemia. This compensation to a diabetogenic stimulus has been shown to be accounted for by progressive morphologic changes in the pancreas which reflect increased insulinogenesis. Thus, in the rabbit there is initially degranulation and increased mitotic activity of the β cells. With further progression of treatment, neogenesis of β cells from primitive precursors can be traced from pancreatic duct epithelium which results eventually in marked islet hyperplasia.

In contrast to the rabbit and guinea pigs, no α cell changes were observed in the dogs after prolonged glucagon administration. This may be due to the fact that the amount of glucagon available for use in this species was insufficient or perhaps species difference may be a factor in determining the effect of glucagon on the α cells.

In the two partially depancreatized cortisone and glucagon-treated dogs there was transient mild to moderate hyperglycemia with increased urinary glucose output. However, in the third animal, which was 80 per cent partially pancreatectomized, a severe and sustained diabetes developed after thirty-one days of treatment and the pancreas resembled pancreases found in other forms of metadiabetes.[29,39,47–55] Identifiable β cells were no longer present and there was marked fibrosis of the pancreatic remnant. In addition, there was marked vacuolization of duct epithelium and these vacuoles frequently contained glycogen deposits. The problem arises whether the glucagon administration was responsible for the destruction of the β cells, or whether this might have occurred in any case because of the comparatively extensive partial pancreatectomy or because of the administration of 1 mg./kg. daily of cortisone. However, in previous work, severe diabetes was induced only in a small percentage of 80 per cent partially pancreatectomized animals by dosages of cortisone of up to 10 mg./kg. daily.[48] Furthermore, hyperglycemia did not develop in the three partially pancreatectomized control animals treated with 1 mg./kg. of cortisone alone. The development of permanent diabetes with β cell destruction in the canine has been reported after growth hormone,[29,40,48–52] cortisone,[53] thyroid extracts[51,54] and glucose infusion,[29] as well as after partial pancreatectomy alone.[55] It is the hyperglycemia which is considered to be the common noxious agent responsible for the β cell destruction. If glucagon, which produces hyperglycemia, could also definitely be shown to cause permanent diabetes, this viewpoint would be reinforced. The results of the present experiments are equivocal in this regard since the dog which became diabetic was the one in which four fifths of the pancreas had been removed and in which the residual organ showed fibrosis.

SUMMARY

A study was conducted on the effect of long continued administration of glucagon with or without cortisone on the blood and/or urinary glucose and on the pancreatic morphology of rabbits, guinea pigs and dogs. Glucagon alone in rabbits and guinea pigs produced only a moderately increased blood sugar concentration or glycosuria. On the other hand, glucagon with cortisone produced in rabbits a severe hyperglycemia which generally tended to improve after approximately six weeks of treatment. Morphologically, most glucagon treated rabbits and guinea pigs showed a reduction in the number of identifiable α cells. In many instances partly degranulated or poorly staining α cells were observed as well as occasional groups of nuclei with ill-defined cytoplasm which resembled the nuclei or identifiable α cells and which were thought to be degranulated α cells. These findings suggest that glucagon is a hormone and that it is derived from this cellular system. The guinea pigs showed only very moderate degranulation of β cells while in the rabbits marked β cell degranulation and glycogen infiltration of duct epithelium and of β cells was observed. Furthermore, in rabbits treated for long

periods there was β cell hyperplasia and neogenesis of these cells from ductular epithelium via primitive β cells could be traced.

In the dogs no α cell changes were observed. In two 50 per cent pancreatectomized dogs no or very mild transient morning hyperglycemia occurred. In a third animal, severe prolonged diabetes developed which was associated with loss of β cells. In this animal 80 per cent of the pancreas had been removed at surgery and there was fibrosis of the remnant organ. This may possibly be another example of the production of pancreatic β cell destruction in the canine during prolonged hyperglycemia. However, due to inadequate supplies of glucagon additional experiments to verify this finding could not be undertaken.

SUMMARIO IN INTERLINGUA

Studios Relative al Action Diabetogene de Glucagon e al Sito de Su Origine

Esseva interprendite un studio relative al effecto del prolongate administration de glucagon—con o sin cortisona—super le concentration sanguinee e/o urinari de glucosa e super le morphologia pancreatic in conilios, porcos de India, e canes. Glucagon sol produceva in conilios e porcos de India solmente un moderate augmento del concentration sanguinee de sucro o de glycosuria. Del altere latere, glucagon in combination con cortisona produceva in conilios sever grados de hyperglycemia que tendeva generalmente a meliorar se post approximativemente sex septimanas de tractamento. Quanto al morphologia, le majoritate del conilios e porcos de India tractate con glucagon monstrava un reduction del identificabile cellulas alpha. In multe casos, cellulas alpha partialmente disgranulate o imperfectemente acceptante le colorante esseva observate. Esseva etiam notate le presentia sporadic de gruppos de nucleos con mal definite cytoplasma que resimilava le nucleos de identificabile cellulas alpha e que esseva considerate como disgranulate cellulas alpha. Iste constatationes pare indicar que glucagon es un hormon e que illo es derivate ab iste systema cellular. Le porcos de India monstrava solmente levissime grados de disgranulation de cellulas beta, durante que le conilios exhibiva marcate grados de disgranulation de cellulas beta e de infiltration de glycogeno in le epithelio del ductos e in le cellulas beta. In plus, in conilios tractate durante prolongate periodos de tempore il occurreva hyperplasia de cellulas beta, e neogenese de iste cellulas ab le epithelio ductular via primitive cellulas beta poteva esser observate.

In canes, nulle alteration del cellulas alpha esseva observabile. In duo canes con pancreatectomia de 50 pro cento, nulle o levissime hyperglycemia transitori occurreva in le matino. Un tertie animal disveloppava sever e prolongate diabete que esseva associate con le perdita de cellulas beta. In iste animal, 80 pro cento del pancreas habeva essite excidite chirurgicamente, e in le resto del organo fibrosis esseva presente. Isto es possibilemente un exemplo additional del effectuation de un destruction del cellulas beta del pancreas in canes con hyperglycemia prolongate. Tamen, in consequentia de inadequate quantitates de glucagon disponibile pro iste studio, experimentos additional pro verificar le mentionate constatation non poteva esser interprendite.

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Pancreatic Islets in Mice with the Obese-hyperglycemic Syndrome

Lack of Effect of Carbutamide

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The hereditary obese-hyperglycemic syndrome is a Mendelian recessive condition which has been intensively studied in the last few years. In particular, mice with this syndrome are grossly obese (weights up to 115 gm. have been recorded), hypercholesterolemic, show high blood glucose levels generally further increased by the administration of growth hormone,[1] an increased lipogenesis even when fasted,[2] increased glycogen turnover,[3] increased liver phosphorylase,[4] and a number of other nutritional, endocrine and behavioral idiosyncrasies.[5] Of particular interest from the etiological point of view is the fact that the islets of Langerhans are grossly hypertrophied,[6-8] that the insulin content of the pancreas is increased,[7] that the insulin-like activity of their serum is greater than that of the nonobese animals,[9] that diethyldithiocarbamate, which Kadota and Midorikawa found to be alphacytotoxic,[10] decreases blood sugar and eliminates the hyperglycemic reaction to growth hormone[11] and that the pancreatic glucagon content appears increased after growth hormone administration.[12] It has also been found that carbutamide did not correct the defects in carbohydrate metabolism[13] and in fat metabolism[14] found in these animals.

It is obviously essential to the understanding of the syndrome to gather as accurate histologic data as possible. The lack of effect of carbutamide in a form of diabetes which is not due to a deficiency of insulin is of relevance to the formulation of a hypothesis concerning the mode of action of this substance and treated mice appeared worth including in this study.

MATERIALS AND METHODS

Twenty-one obese-hyperglycemic mice and twenty-four sibling controls were studied. The animals were either two or four months old at the beginning of the experiment (four or six months old when sacrificed). To simplify the presentation of results, the younger group is referred to as "young" in the tables, the older group as "adult," though by the time the animals were sacrificed all were relatively young adults. Life expectancy in these mice is of the order of two to three years in the nonobese animals, eighteen months to two years for the obese. Males and females were equally or nearly equally distributed among the various groups. As no differences could be detected in the appearance or the quantitative characteristics of the pancreas between males and females, results for the two sexes were not reported separately. All animals were maintained on Purina chow for three weeks, then carbutamide was administered in the diet of the treated animals for seven weeks (2.5 gm. carbutamide being uniformly mixed in each kilogram of diet).

At the end of the experimental period, blood glucose levels were determined by the method of Somogyi-Nelson.[15] Animals were sacrificed by decapitation. The whole pancreas was carefully dissected from the spleen, the stomach and the duodenum, fixed in Bouin's solution, embedded in paraffin and serially sectioned in 30-micron sections with one section out of five being retained. Some sections 5 microns thick were obtained from among the discarded sections.

The thicker sections were stained with hematoxylin-eosin, the thin with Gomori's chromium hematoxylin.[16] Islet tissue volume was determined by two methods. In the "volumetric" method the volume of each islet is approximated to that of the sum of cylinders having as base the surface (S) of the cross-section and as height the interval between two cross-sections (0.03 mm. × 5). All islets present in one slide (out of five) are drawn at a linear magnification of 160 and their area of the cross-section is measured with a planimeter. On the basis of these assumptions the total islet volume is therefore given by the formula:

$$V = \frac{S(\text{mm.}^2) \times 0.03 \text{ (mm.)} \times 5}{160^2}$$

The other method used was Tejning's "numerical" method[17] which yields estimates not only of the total volume but also of the number of islets, the distribution

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by size and the contribution of each size class to the total islet volume. The accuracy of these two methods has been evaluated elsewhere.[38] In this connection it must be noted that each continuous or contiguous group of islet cells was counted as one islet without any attempt made to estimate the number of coalesced islets. This fact may be of particular importance in the interpretation of islet number in the obese, where the total number of islet cells is increased severalfold, while the total mass of the pancreas is only slightly increased.[6]

The differential cell counts were established by counting under immersion 2,000 islet cells in 5-micron sections stained by the Gomori technic.[10] Beta granules were stained with chromium hematoxylin for eight minutes at 56° C., alpha granules with phloxine. Degranulation when present rendered differential counts more difficult. Any cell with a blue granule was diagnosed as a beta cell; with a red granule as an alpha cell. The facts that alpha cells are smaller and usually located at the periphery of the islets were taken into consideration.

RESULTS

I. Islet tissue in untreated mice.

1. *Quantitative results.* Table 1 gives the total islet volume determined by both the volumetric and the numerical methods in young and adult obese-hyperglycemic mice and their littermate controls. The two methods give results in excellent agreement with each other. Obese mice have about six times as much islet tissue as the nonobese. In the obese animals, the number of islets is slightly lower than in the nonobese (table 2).

TABLE 1

Volume of pancreatic islet tissue per animal (mm.^{3}) as determined by the volumetric (V) and numerical (N) methods in obese-hyperglycemic mice and lean littermates, untreated and long-term treated with carbutamide (BZ-55)

Method		Nonobese young		Obese young		Nonobese adult		Obese adult	
		Vol.	Num.	Vol.	Num.	Vol.	Num.	Vol.	Num.
Untreated mice		0.505	—	1.626	—	0.765	0.799	3.060	2.736
		0.433	—	5.733	5.132	0.770	0.710	5.058	4.303
		0.311	0.301	2.923	2.978	0.502	0.485	5.481	4.465
		0.598	0.551	3.053	2.708	0.625	0.643		
		0.487	0.503	2.769	2.623				
		0.484	—	1.633	1.769				
		0.472	0.420	3.020	2.787				
		0.704	0.698	2.707	2.480				
	Mean	0.499 ±0.115	0.493	2.933 ±0.402	2.920	0.666 ±0.128	0.640	4.533 ±1.293	3.835
Carbutamide-treated mice		0.793	0.786	2.481	2.176	0.960	0.925	4.303	4.551
		0.642	0.772	6.184	6.517	0.729	0.747	5.140	5.622
		0.683	0.682	3.859	4.130	0.816	0.840	5.637	5.349
		0.677	0.750	1.448	1.355	0.534	0.558		
		0.858	0.734	4.384	4.526	0.761	0.736		
		0.748	0.696	3.832	3.547				
		0.825	0.871	1.271	1.132				
		0.747* ±0.052	0.713	3.351 ±1.745	3.340	0.760 ±0.154	0.761	5.027 ±0.674	5.174

Figures following ± are standard deviations. Because of the similarity of results for each mouse by the two methods, standard deviations have been calculated only for the volumetric method.
*Difference between treated group and control group significant at 5 per cent level.

TABLE 2

Average number of pancreatic islets in obese-hyperglycemic mice and their lean littermates. Comparison between treated and untreated animals

	Nonobese young	Obese young	Nonobese adult	Obese adult
Untreated mice	547±258	467±167	729±501	589±158
Carbutamide-treated mice	1,014±137*	619±218	925±293	680±205

Figures following ± are standard deviations.
*Difference between treated group and control group significant at 5 per cent level.

The difference is not significant and may be an artifact because of the confluence of several islets due to the crowding of hypertrophied islets in the obese animals.

The classification of islets according to size by Tejning's method shows that in nonobese mice the islets are more numerous and they are smaller. In obese mice, islets of intermediary size are the most numerous (figure 1). Furthermore, while in nonobese animals intermediary size islets contribute the greater part of the total islet tissue volume, the larger proportion of islet tissue in obese-hyperglycemic mice is contributed by the big islets (figure 2).

Table 3 shows that the relative increase in number of beta cells in the obese animals is larger than the relative increase in alpha cells so that the ratio of beta to alpha cells is greater in the obese mice.

2. *Appearance of the islets and cells.* In accordance with previous descriptions[6-8] it was observed that the beta cells were degranulated, hypertrophied and showed numerous mitotic figures (figures 3 and 4). The alpha cells were also hypertrophied and appeared degranulated though the difference compared to the nonobese was less definite than in the case of the beta cells. The islets in the obese were strikingly hyperemic. Neoformation of islet tissue seemed to take place at the end of the secretory ducts.

We interpreted the appearance of certain sections as suggestions that some acinar cells were differentiating into islet cells.

II. Effect of carbutamide.

The daily consumption of carbutamide during the treatment period was of the order of 13 mg. per day for the nonobese (0.5 gm. per kg. of body weight) and 17 gm. per day for the obese (0.3 gm. per kg. body weight). These doses had no effect on the food intake or the weight of treated animals (table 4). A small but significant increase in liver weight and blood glucose levels was observed in the nonobese mice. Liver hypertrophy and lack of improvement of the "diabetes" were observed in the obese animals.*

Table 1 shows that the volume of pancreatic islets was somewhat greater in all groups of animals, obese and nonobese, four months and six months old, treated with carbutamide. However, only the treated young nonobese mice showed differences from their controls significant at the 5 per cent level. Carbutamide treatment also seemed to increase islet numbers (table 2); however, only the young nonobese animals differed from their control at this level of significance. Figures 1 and 5 show that in this group the increase in number of islets is predominantly in the classes of small islets.

*Carbutamide does not alleviate the hyperglycemia of obese-hyperglycemic mice.[13]

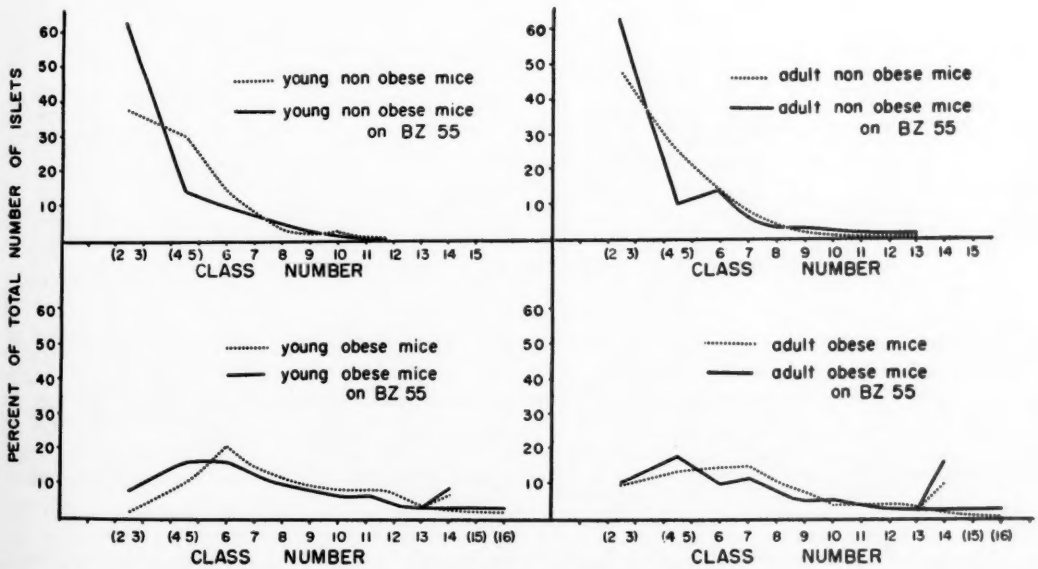


FIG. 1. Frequency distribution of the volumes of individual islets. The value given in ordinate is the percentage of the total number of islets of the size indicated by the abscissa. The value given for class 14 is representative not only of this class but also of the classes corresponding to larger islets.

TABLE 3

Percentage of α cells in the pancreatic islets of obese-hyperglycemic mice and their lean littermates in untreated animals and animals long-term treated with carbutamide (BZ-55)

	Nonobese young	Obese young	Nonobese adult	Obese adult
Untreated mice	13.9	10.1	19.9	3.8
	20.6	11.2	17.9	5.8
	17.5	12.0	17.3	3.3
	17.3	4.3	18.3	5.2
	10.7	4.6		
	18.9	9.4		
	15.2	8.8		
	11.3	5.0		
	28.3			
	17.1±5.4	8.2±3.1*	18.4±1.2	4.6±1.2*
Carbutamide-treated mice	13.8	16.7	14.7	11.9
	15.6	8.6	11.5	3.5
	11.7	6.4	13.7	1.3
	9.8	9.4	13.3	
	20.7	2.2	26.8	
	8.4	14.8		
	15.0	15.1		
	8.7			
	13.0±4.2	10.5±5.3	16.0±6.1	5.6±5.6

Figures following ± are standard deviations.
*Difference between untreated obese group and corresponding untreated nonobese group significant at 5 per cent level.

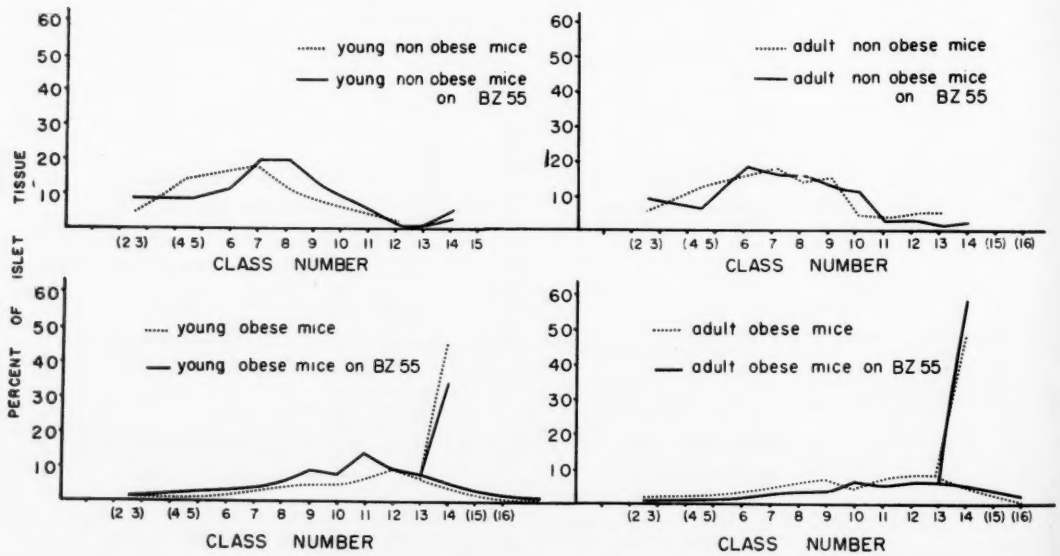


FIG. 2. Percentage of total islet tissue corresponding to islets of a given volume. The value given for class 14 is representative not only of this class but also of the classes corresponding to larger islets.

In the obese mice the percentage of islets of the various sizes is similar in treated and untreated animals. In terms of contribution of the various islets to total volume, the increase in islet volume in treated nonobese younger mice is due to the increase in the size of the large islets which more than makes up for the decreased volume of islets of intermediary size. In the other groups, the volume distribution does not appear to be affected by carbutamide (figure 2). The relative proportion of islet cells of different types did not appear to

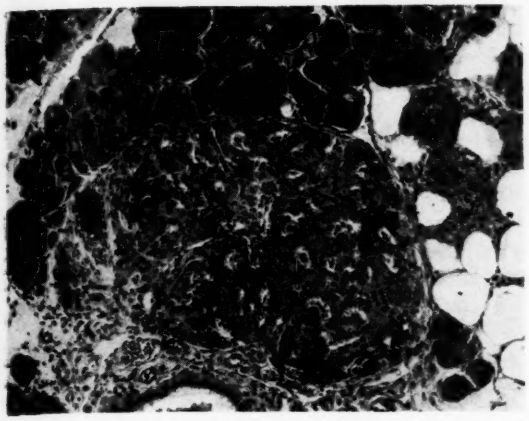


FIG. 3. Pancreatic islet of an obese-hyperglycemic mouse (× 62.5).

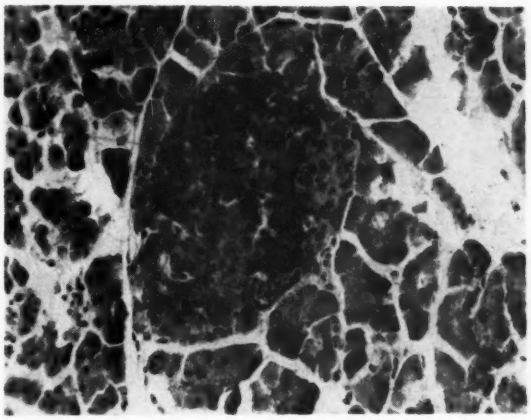


FIG. 4. Pancreatic islet of a nonobese littermate (× 125).

TABLE 4

Average daily food intake, weight increase (percentage over weight at beginning of experimental period), final body weight, liver weight, and blood sugar level of obese-hyperglycemic mice and lean littermates untreated and treated for forty-nine days with carbutamide

Type and number of mice			Food intake gm./day	Wt. increase (per cent)	Body wt. gm.	Liver wt. per cent of total wt.	Blood sugar level mg. per 100 ml.
Nonobese littermates							
Untreated	young	(8)	5.3	16	26		89
	adult	(4)	5.1	0	28		89
	total	(12)	5.2±1.4	—	27	4.9±0.3	89±21
Carbutamide-treated	young	(7)	4.9	14	24		112
	adult	(5)	6.0	5	27		133
	total	(12)	5.2±1.4	—	26	5.8±0.6†	120±31*
Obese-hyperglycemic							
Untreated	young	(8)	6.3	35	56		236
	adult	(3)	6.5	16	62		193
	total	(11)	6.4±1.4	—	58	6.0±0.9	223±118
Carbutamide-treated	young	(7)	5.9	32	55		202
	adult	(3)	6.6	9	64		204
	total	(10)	6.1±1.2	—	58	7.1±1.0*	203±57

Figures following ± are standard deviations.
*Differences between treated group and control group significant at 2 per cent level.
†At 1 per cent level.

be affected by carbutamide. Carbutamide treated nonobese mice showed occasionally some degree of degranulation of beta cells. No degenerative changes were observed in either beta or alpha cells. The appearance of islets in obese animals was not affected by carbutamide treatment: Beta cells were still degranulated, mitotic figures were as numerous, and the picture of hyperemia and neoformation of islets was not modified.

DISCUSSION

The use of quantitative determinations confirms the existence of a considerable degree of hypertrophy of islet tissue in the hereditary obese-hyperglycemic syndrome. Furthermore, these determinations show that this hypertrophy is due much more to an increase in the size of the islets than to an increase in their number. While the absolute number of both alpha and beta cells is increased, that of the beta cells is increased more in proportion; for example in the four-month-old group, the obese mice have about six times as much islet tissue as the nonobese with a proportion of 8 per cent of alpha cells instead of 17 per cent. In other words, while they probably have about six times as many beta cells they have only about three times as many alpha cells.

It is interesting to note that many symptoms seen in obese-hyperglycemic mice are also present in obese

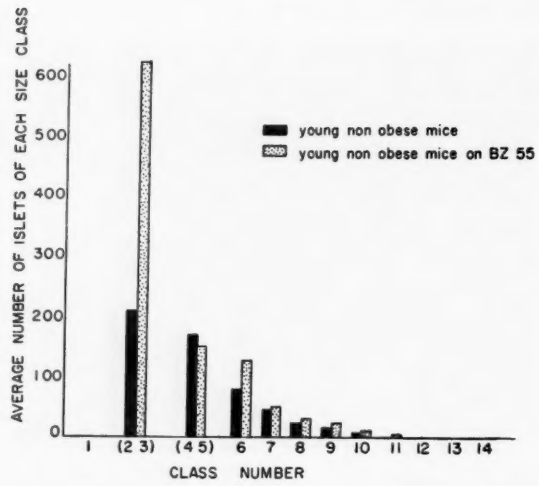


FIG. 5. Average distribution of islet size in young nonobese mice untreated or long-term treated with carbutamide.

diabetic patients, e.g., obesity, hypercholesterolemia and decreased insulin sensitivity. However, there are some important differences, such as the fact that there is nearly always a diminution in the number of beta cells in diabetic human beings.[19,20]

The interpretation of the present results from the point of view of the pathogenesis of the syndrome is not obvious. It has been hypothesized[5,11] that the primary lesion in the obese-hyperglycemic syndrome was the hypersecretion of both beta cells (insulin) and alpha cells (glucagon).

Supporting this possibility were the findings of increased pancreatic[7] and circulating[6] insulin-like activity on the one hand and increased liver phosphorylase activity,[4] liver glycogen turnover[3] and pancreatic glucagon after growth hormone administration[12] on the other. While evidence supporting increased insulin secretion is thus direct, that supporting increased glucagon secretion is only circumstantial. While the present findings are definitely in disagreement with the hypothesis of Ferner[21] that the alpha/beta ratio determines the extent of hyperglycemia, in any form of diabetes, the lack of direct data on glucagon secretion prevents the determination of whether the absolute increase in alpha cells or the relative decrease in proportion of alpha cells is a determining factor in the etiology.

The lack of effect of carbutamide in the obese-hyperglycemic mice is of interest in view of the hypothesis that such substances may be active in forms of diabetes not characterized by absence of circulating insulin. The most likely explanation is that the pancreas of obese-hyperglycemic mice already produces and releases all the insulin of which it is capable so that additional stimulation is without effect.[13] In nonobese mice, the acute administration of carbutamide causes temporary hypoglycemia.[18] Long-term administration causes a slight increase in blood glucose levels and also, particularly in younger animals, islet hypertrophy with production of new small islets. These findings in the normal mouse are not unlike those in the normal rat where long-term carbutamide administration causes a decrease in rate of glucose utilization,[22] some loss of beta granules and islet hypertrophy.[20,23] It has been reported that certain strains of rats react to long-term carbutamide treatment by an increase in blood sugar levels.[24] It has also been found that the glucose tolerance of partially depancreatectomized rats was more severely impaired when the animals were treated with tolbutamide.[25] It must be stressed, however, that in none of these experiments was there any evidence of damage to the beta cells secondary to sulfonylurea treatment.

SUMMARY

1. Quantitative determination of pancreatic islet tissue in mice with the obese-hyperglycemic syndrome shows considerable hypertrophy due to an increase in the size of the islets. The number of beta cells is increased to a greater extent (approximately six times) than the number of alpha cells (approximately three times).

2. The "diabetes" is not cured by long-term treatment with carbutamide.

3. In nonobese siblings carbutamide, while hypoglycemic in short-term treatment, causes in long-term treatment a slight increase in blood sugar levels and islet hypertrophy with production of new islets.

SUMMARIO IN INTERLINGUA

Insulas Pancreatic in Muses con le Syndrome de Obesitate e Hyperglycemia: Absentia de Effecto de Carbutamido

1. Le determination quantitative de tissu del insulas pancreatic in muses con le syndrome de obesitate e hyperglycemia revela considerabile grados de hypertrophia in consequentia del augmentate dimensiones del insulas. Le numero del cellulas beta es augmentate al sextuple, illo del cellulas alpha solmente al triple.

2. Le "diabete" non es curate per tractamentos prolongate con carbutamido.

3. In non-obese fraternos, carbutamido—que es de effecto hypoglycemic in curte cursos de tractamento—causa in prolongate cursos un leve augmento del nivellos de sucro in le sanguine e hypertrophia del insulas con production de insulas additional.

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EDITORIAL

INSULIN AND PINOCYTOSIS

In 1931 W. H. Lewis[1] coined the word pinocytosis, meaning "cell drinking," to describe the phenomenon whereby a cell engulfs some of the fluid medium bathing it. This process resembles phagocytosis but differs from it in that a liquid droplet rather than a solid particle is taken into the cell. The droplet engulfed is surrounded by a membranous envelope which was derived from the surface membrane of the cell. Pinocytosis was observed by Lewis to occur in tissue culture cells derived from both normal[1] and malignant tissues.[2] The process has been studied in amoebae[3–5] and more recently pinocytosis has been observed in various mammalian tissues[6,7] (cf. also Bennett[8]). In the latter case the process occurs on such a micro scale that it can only be seen with the aid of the electron microscope.

It has now been found that the addition in vitro of insulin to adipose tissue (epididymal) of the rat stimulates the process of pinocytosis.[9] Concomitantly, glucose is rapidly taken into the cells and converted to fat.[10,11] Observations with the electron microscope show that rapid and pronounced morphologic changes occur in adipose tissue after a brief period of incubation in solutions containing 0.04 γ of insulin per milliliter. The plasma membranes of the adipose cells become invaginated at many sites to form minute fingerlike indentations. Numerous tiny, membrane-bounded vesicles are also present and arranged in relationship to the plasma membrane in such a way as to suggest that their formation occurred when a recessed fold was pinched off. Deeper in the cytoplasm, especially in specimens that had been incubated a longer time, numerous large, smooth membrane-enveloped vesicles are seen. In the virtually two-dimensional sections of tissue these vesicles appear to form a discontinuous reticulum from the plasma membrane and its infoldings deep into the interior of the cytoplasm. Occasionally, where the plane of section was fortuitous, some continuous canalicular channels of reticulum attached to the plasma membrane at one end are found. Finally, in these incubated specimens the cytoplasm has lost much of its granularity and small lipid droplets are frequently found. In control specimens, incubated without insulin for identical periods of time, indentations and vesicles in the plasma membrane were sparse at best and no membranous system of cytoplasmic reticulum occurred.

The formation of vesicles by the process of micropinocytosis provides a mechanism whereby the fluid surrounding a cell may enter it and carry with it certain small molecules such as glucose. It is possible that large protein molecules in the fluid may be screened out by this process due to the small diameter of the invaginations or channels which become pinched off to form the vesicles. Thus the diameter of the channels formed may limit the size of the molecules to be admitted. At any rate, the droplets of surrounding medium that are carried into the cell are enclosed by an envelope which is derived from the plasma membrane of the cell, a membrane which is poorly permeable to glucose in the absence of insulin. This fact poses a problem in connection with the escape of glucose from these vesicles into the cytoplasm of the cell. An answer to this question has been sought by Chapman-Andresen and Holter[5] in their work with amoebae. These investigators have followed the uptake of radioactive glucose into amoebae during pinocytosis by the use of radio-autographs. They have shown that when a vesicle is formed it contains some of the radioactive glucose present in the medium surrounding the amoebae. Shortly after formation of the vesicle the radioactive glucose can be seen to have leaked into the cytoplasm. It would thus appear that the envelope surrounding the vesicle acquires permeability properties to glucose which are quite different from that of its plasma membrane precursor.

Whether the process of vesicle formation alone is sufficient to account for the very rapid rate at which glucose enters the cell on stimulation by insulin is open to question. On the one hand it is difficult to arrive at a reasonably accurate estimate of the amount of pinocytosis going on and hence at the amount of glucose which enters in this manner. On a morphologic basis the idea is feasible and appealing. On the other hand, in the presence of insulin the amount of glucose consumed by adipose tissue in a single hour is equal to that contained in a volume of medium which is three to five times the volume of the tissue. Since glucose consumption continues unabated for at least six hours in the experiments run in vitro, this would require a sizable intake of fluid if vesicle formation is to account for the admission of all the glucose that is consumed. It is thus possible that the process of pinocytosis brings about structural changes in the cell which facilitate

the entrance of glucose and other small molecules by means other than vesicle formation. For example, the deep invaginations which are not pinched off may acquire permeability properties similar to those of the vesicles. Or it is possible that alterations in the permeability of the noninvaginated surface membrane are promoted so that certain molecules gain admission more readily. In this regard it should be reiterated that the process of vesicle formation in pinocytosis results in a loss of surface membrane which would require the cell either to replace it or make some other adjustment.

The mechanism by which insulin stimulates pinocytosis is unknown. However, it has been shown[12,13] in amoebae that certain proteins stimulate pinocytosis. Brandt[13] has labeled one of these proteins, serum γ-globulin, with a fluorescent dye in order to visualize the process and has shown that the first step is the adsorption of the protein to the cell membrane or plasmalemma. Invagination of the membrane then occurs at the site where the protein is adsorbed. The protein which initiated the process is thus carried into the cell and it can be seen attached to that part of the membrane which has become the envelope surrounding the vesicle. Indeed, it is not impossible that this adsorbed protein is responsible for the alteration in the permeability properties of the membrane.

Insulin may be similarly adsorbed on the cell membrane as a first step in its initiation of pinocytosis. Such an adsorption would find support in the observation of Stadie and his colleagues[14] on the fixation of insulin to diaphragm muscle. These workers observed that when diaphragm was briefly dipped into insulin solutions, removed, thoroughly washed, and then placed in a glucose-containing medium, an enhanced glucose uptake occurred. Similar effects can be observed with adipose tissue. The size of the vesicle that is formed might be a function of the size of the protein that is adsorbed. In the case of amoebae where serum γ-globulin with a molecular weight of 180,000 is the initiating agent the vesicles formed are large enough (2-10 μ) to be seen with the light microscope. In the case of insulin with a molecular weight of 6,000 the vesicles formed have diameters in the range 20-50 mμ and hence are only visible with the electron microscope. It will be of interest to learn whether other hormones possess the property of initiating pinocytosis, or perhaps inhibiting it. Those hormones which are proteins of low molecular weight and have an action on a specific target tissue should be of particular interest.

As a result of their studies, Levine and his associates[15] have proposed that insulin acts by enhancing the entrance of hexoses into cells. The observations of Park[16] and others have confirmed and extended Levine's original observations. The finding that insulin stimulates the process of pinocytosis lends further support to the thesis that this hormone regulates the permeability of the cell to glucose and suggests a mechanism by which this may be accomplished. Furthermore it suggests that hormone stimulation of pinocytosis at a submicroscopic level may play an important role in the so-called active transport of, and the permeability of cells to, certain materials.

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James F. Hart
1886—1959

Frederick W. Williams, M.D., New York

James Finlay Hart, M.D., a member of this Association for many years, died on Feb. 24, 1959, after a long illness. He was born on Dec. 19, 1886, in Fordham, New York, the son of Elizabeth Margaret Finlay and Michael Hart.

His early education was obtained in the local public schools, where he showed outstanding ability and received many awards of merit. He worked in his free time while he attended high school, helping to support his widowed mother.

Although he did not have sufficient funds of his own to go to college, he won enough scholarships to obtain a premedical education at Cornell University. He was awarded his medical degree by Syracuse University College of Medicine in 1911.

After becoming assistant to a prominent physician at St. Elizabeth's Hospital, Dr. Hart took four months' training at Bellevue Hospital. He also spent four months at Misericordia Hospital and served as Assistant House Surgeon at The Lying-In Hospital.

During his long and busy life, which he devoted to medicine and to humanitarian endeavors, he was appointed Adjunct Professor of Medicine at New York Polyclinic Medical College and Hospital, Attending Physician and Director of Metabolism at Midtown Hospital, Associate Clinical Professor of Medicine at New York Medical College, president of the Clinical Society of the New York Polyclinic Medical School and Hospital, president of the Bronx Medical Association, a Life Member of the New York Academy of Medicine and chairman of the Clinical Society of Midtown Hospital.

Devoting himself to the practice of internal medicine, Dr. Hart contributed to medical literature more than fifty articles dealing with clinical and pathological subjects. His interest in diabetes was great.

He was intensely interested in Camp NYDA, sponsored by the New York Diabetes Association, from its very inception, and did much to develop it. Frequently Dr. Hart gave talks on Camp NYDA over the radio, hoping to arouse the interest of potential contributors to the camp fund, to enable diabetic boys and girls of all creeds and races to attend summer camp. In 1955 Dr. Hart was elected president of the New York Diabetes Association.

During the last years of his life he devoted much time to research in youth problems, working to complete a book on juvenile delinquency as it is observed by the physician. Throughout his medical practice, he always held high the ideals of the family doctor, even though he limited his practice to internal medicine—predominantly diabetes and heart disease.

Through his hospital affiliations, his contributions to the medical literature, and his wide and extensive practice in his fields of interest, he will always remain highly regarded by those who have read his works, respected by those of the medical profession who were fortunate enough to know him personally, and revered by the ones who came under his care professionally. Dr. Hart was a man who was truly a patient's doctor, and will long be remembered as a man amongst men.

BOOK REVIEW

NUTRITIONAL DIAGNOSIS. *By Grace A. Goldsmith. M.D. $5.50, pp. 164, Charles C Thomas, Publisher, Springfield, Illinois.*

There has been a real need for a text presenting modern concepts of evaluation of the nutritional status of individuals. Dr. Goldsmith has succeeded in writing a clear, concise text from the clinical point of view which should be of immense value to the general practitioner and medical students.

The chapter on "General Observations" is unequalled in any of the currently available texts on the subject. The outline of the physical signs commonly associated with nutritional disturbances is clear, concise and useful. There is a table outlining the signs and symptoms suggestive of deficiencies of certain nutrients. This represents a tremendous amount of work and is unique. The table indicating the laboratory procedures useful in nutritional diagnosis and the limits of the normal will simplify the life of the clinician who wants a quick reference.

The main text of the book is writen clearly and in a didactic style that lends itself to efficient utilization.

ABSTRACTS

Adlersberg, David; and Eisler, Lisbeth (Mount Sinai Hosp., New York City, N.Y.): CIRCULATING LIPIDS IN DIABETES MELLITUS. J. A. M. A. *170*:261-65, July 11, 1959.

Many patients with diabetes mellitus present abnormalities in levels of circulating lipids in absence of keto-acidosis and renal disease. These are characterized by elevation of the levels of serum fatty acids and triglycerides with only moderate or no increase in the levels of serum cholesterol and phospholipid. They are mainly related to two factors: decompensation of deranged carbohydrate metabolism (lack of insulin) and the presence of vascular complications. Maintenance of normal levels of circulating lipids should be added to the criteria of management of diabetes mellitus. Observation of this parameter of diabetes control may improve the prognosis of patients with diabetes, especially in regard to the complications. Adequate compensation of deranged carbohydrate metabolism appears to be an important prerequisite for adequate control of lipid metabolism. B.F.K.

Albanese, A. A.; Higgons, R. A.; Lorenze, E. J.; and Orto, L. A. (St. Luke's Convalescent Hosp., Greenwich, Conn.): EFFECT OF DIETARY PROTEINS ON BLOOD CHOLESTEROL LEVEL OF ADULTS. Geriatrics *14*:237-43, April 1959.

Blood cholesterol levels of aging human adults are not necessarily influenced by over- or undernutrition in terms of body weight. Increases in protein intake by supplementation with a milk-protein concentrate were more frequently associated with an increase, rather than a decrease, in blood cholesterol levels. S.S.

Allwood, M. J.; Hensel, H.; and Papenberg, J. (St. Thomas's Hosp. Med. School, London, S.E. 1, England): MUSCLE AND SKIN BLOOD FLOW IN THE HUMAN FOREARM DURING INSULIN HYPOGLYCAEMIA. J. Physiol. *147*:269-73, Sept. 2, 1959.

Blood flow was recorded simultaneously in skin and muscle of the human forearm during insulin hypoglycemia, using thermal-conductivity meters. Following an initial decrease of short duration, cutaneous blood flow increased rapidly during hypoglycemia to twice the resting value. Muscle blood flow increased during the same time to 175 per cent of the resting level. Comparison with the response to intravenous adrenalin indicates that the increased muscle flow during this time may not be due solely to adrenalin release. G.A.W.

Appelman, David H. (Diabetic Clin., Jewish Chronic Disease Hosp., Brooklyn, N.Y.): DIABETIC DETECTION. Am. Pract. & Digest Treat. *10*:1499-1500, September 1959.

This is a discussion of the various types of oral glucose tolerance tests in use, including the cortisone-glucose tolerance test. The concentration of diabetic screening upon relatives of diabetics is proposed as an efficient substitute for mass screening programs. S.B.B.

Ball, Eric G.; Martin, Donald B.; and Cooper, Octavia (Dept. of Biol. Chem., Harvard Med. Sch., and Baker Clinic Res. Lab., Boston, Mass.): STUDIES ON THE METABOLISM OF ADIPOSE TISSUE. I. THE EFFECT OF INSULIN ON GLUCOSE UTILIZATION AS MEASURED BY THE MANOMETRIC DETERMINATION OF CARBON DIOXIDE OUTPUT. J. Biol. Chem. *234*:774-80, April 1959.

The release of carbon dioxide from fat in the Warburg apparatus was utilized to measure the effect of insulin on rat adipose tissue. Carbon dioxide release was accelerated by addition of insulin to the medium, indicating fat synthesis from glucose. A.R.C., JR.

Bishop, Jonathan S.; and Marks, Paul A. (Dept. of Med., College of Physicians & Surgeons, Columbia Univ., New York, N.Y.): STUDIES ON CARBOHYDRATE METABOLISM IN PATIENTS WITH NEOPLASTIC DISEASE. II. RESPONSE TO INSULIN ADMINISTRATION. J. Clin. Invest. *38*:668-72, April 1959.

The carbohydrate metabolism of a group of normal control adults and of adults with neoplastic disease was studied by an intravenous glucose tolerance test (25 gm. in four minutes) and by an insulin tolerance test (0.1 unit/kilogram body weight). These two tests were performed in succession. The patient response was measured by mathematical rate constants expressive of rate of disappearance of blood glucose. Both tests showed decreased responsiveness of patients with neoplastic disease to utilization of glucose by the action of either endogenous or exogenous insulin. S.B.B.

Bloom, Arnold (Whittington Hosp., London, England): REMISSION IN DIABETES. Brit. M. J. *2*:731-34, Oct. 17, 1959.

Five hundred and fifty newly diagnosed diabetics were surveyed at the diabetic unit, Whittington Hospital, during the period 1952 to 1957. Of these patients 33.4 per cent were adequately controlled by dietary restriction while a further 26.3 per cent were ultimately controlled by diet alone after an initial treatment with insulin or tolbutamide. The remaining 40.2 per cent needed permanent treatment with insulin or tolbutamide. Insulin was deemed necessary for 318 newly discovered diabetics and of these ninety-two patients were able to maintain control on a simple dietary regimen after gradual withdrawal of insulin. The dangers of unnecessarily prolonged insulin therapy are exemplified in two cases of post-hypoglycemic encephalopathy. J.A.

Bogoch, A.; Davis, T. W.; Jow, E.; and Wrenshall, G. A. (Dept. of Med., Shaughnessy Hosp., Univ. of Brit. Col., Vancouver, B. C.; Charles H. Best Inst., Univ. of Toronto, Ont., Canada): THE CLINICAL RESPONSE AND THE AMOUNT OF INSULIN EXTRACTABLE FROM THE PANCREAS IN DIABETIC PATIENTS TREATED WITH ORAL HYPOGLYCEMIC DRUGS. A REPORT ON FIVE CASES. Canad. M. A. J. *81*: 347-56, Sept. 1, 1959.

A detailed report of five diabetic patients, all over seventy years of age, who were treated with both insulin and sulfonylurea drugs separately. All subsequently died from causes unrelated to the diabetes, and the state of the beta cells and the total amount of extractable pancreatic insulin were determined at postmortem.

Success of therapy with the sulfonylurea drugs was noted in two patients with 25 and 33 per cent insulin content, and lack of success with 0 to 13 per cent. In the remaining

patient with normal insulin content, a sulfonylurea (tolbutamide) was not effective just before death. However, this occurred when relative insulin insensitivity had ensued. S.B.B.

Butterfield, W. J. H.; and Holling, H. E. (Medical Research Council's Clinical Res. Unit, Guy's Hosp., London, S.E. 1, England): PERIPHERAL GLUCOSE METABOLISM IN FASTING CONTROL SUBJECTS AND DIABETIC PATIENTS. Clin. Sc. *18*:147-74, May 1959.

The uptake of glucose by the forearm tissue was determined by measuring simultaneously the arterial and venous blood sugar levels and blood flow. It was found that blood flow through the forearm was so variable that its simultaneous measurement was necessary to determine accurately tissue glucose utilization.

A positive correlation was observed between arterial blood glucose concentration and glucose utilization by the forearm. There appears to be a "threshold" level of sugar above which glucose enters the forearm tissue from the blood and below which glucose passes in the reverse direction. The latter circumstances were generally obtained in fasting severe diabetics with glycosuria. The rate of glucose transport into and out of the tissue was proportional to the difference between arterial glucose and the "threshold" concentration. Insulin appeared to lower the "threshold." For example, a severely ketotic diabetic would show no uptake of glucose by the forearm with an arterial sugar of 300 mg. per cent. With insulin the same patient showed a positive uptake with arterial sugar of 100 mg. per cent.

Stable diabetics show glucose utilization at the same arterial glucose concentrations as normal subjects (100 mg. per cent). The severe or "brittle" diabetic may fail to show glucose utilization by the forearm with arterial sugars of 300 to 400 mg. per cent. Factors regulating the fasting blood sugar and arteriovenous sugar differences of diabetic patients are discussed. J.A.

Chain, E. B. (Depts. of Biochemistry and Chemical Microbiology, Istituto Superiore di Sanita, Rome, Italy): RECENT STUDIES ON CARBOHYDRATE METABOLISM. Brit. M. J. *2*:709-19, Oct. 17, 1959 (Harveian Lecture delivered to the Harveian Society of London on March 18, 1959).

A review of current theories of insulin action and of carbohydrate metabolism in isolated tissues. J.A.

Clinico-Pathologic Conference (Wash. Univ. Sch. of Medicine, Seattle, Wash.): COMPLICATIONS OF DIABETES MELLITUS. Am. J. Med. *25*:627-37, October 1958.

This case report deals with a thirty-seven-year-old insurance broker whose complaints consisted of nausea, vomiting and diarrhea. He had had juvenile diabetes since the age of nine. Impaired vision began two years and edema one year prior to admission. Examination showed hypertension, tachycardia, amaurosis, pallor, lenticular opacities, periorbital edema, absent deep tendon reflexes below the thighs and reduced sensation in the feet, anemia, hyposthenuria, proteinuria, microhematuria, azotemia, hyperglycemia, hypoproteinemia, hypokalemia, incomplete right bundle branch block, and at necropsy atrophy and fibrosis of the pancreas with virtual absence of islets of Langerhans, intercapillary glomerulosclerosis, focal acute and diffuse chronic pyelonephritis, degenerative changes of the retina and optic nerve with subretinal hemorrhage, retinal detachment, perivasculitis, endophthalmitis, hemosiderosis and pannus degeneration; advanced aortic and coronary sclerosis; moderate splenic, renal, and mesenteric atherosclerosis; hypertrophy of the heart and multiple acute ulcerative colitis due to Proteus mirabilis as described by Berge, Sprague and Bennett. L.S.S.

Cohen, Alan S.; Stearns, Norman S.; Levitin, Howard; and Hurwitz, David (Thorndike Memorial Lab., 1st & 2nd Medical Services (Harvard), Boston City Hosp., Dept. of Med., Harvard Med. Sch., & Diabetic Service, Boston City Hosp., Boston, Mass.): STUDIES ON RAUWOLFIA ALKALOIDS IN DIABETIC HYPERTENSIVE PATIENTS. Ann. Int. Med. *51*:238-47, August 1959.

Neither crude (Rauwiloid) nor purified (Reserpine) rauwolfia alkaloids affected the diabetic control or weight in diabetic patients with hypertension and diabetes treated by diet alone or with insulin. S.B.B.

Constam, G. R.; Leemann, W.; and Almasy, F. (Medical Policlinic, consultations for diabetics. Medical clinic & chemical research laboratory of the veterinary faculty, Univ. of Zurich, Switzerland): ABOUT THE EXCRETION OF ZINC IN THE URINE OF DIABETICS. Schweiz. med. Wchnschr. *88*:1103-05, Nov. 1, 1958.

In 1938, Scott and Fisher suggested that zinc might be necessary for the normal storage of insulin in the pancreas. By injecting zinc-binding substances, such as diphenylthiocarbazone, Kadota produced diabetes in rabbits. Wolff and co-workers discovered a transitory increase in the urinary zinc elimination following intravenous injections of diphenylthiocarbazone and also after the injection of alloxan.

Twenty-four-hour specimens of human urine were treated by wet digestion and measured colorimetrically according to the method of Vallee and Gibson.

The urinary zinc elimination of thirty-eight nondiabetics varied from 39 to 892 μg. per twenty-four hours with an average of 337 and the standard deviation of 207.8 μg. per twenty-four hours. Determination of the daily zinc losses in forty diabetics resulted in values from 394 to 5,845 μg. per twenty-four hours with an average of 1,203 and the standard deviation of 914.3 μg. per twenty-four hours. The difference between the mean diabetic and the mean nondiabetic zinc loss, 866 μg. per twenty-four hours, is statistically highly significant. Severe decompensation of the diabetes is usually accompanied by a considerable increase in zinc elimination through the urine. The rise in zinc output apparently does not run parallel to diuresis, glycosuria or ketonuria but just to blood sugar concentration; it seems unrelated to the age of the patient or the estimated duration of the disease. Generally zinc excretion grows smaller with better control of the diabetes.

Whether the increase in urinary zinc elimination in uncontrolled diabetes is the cause or the consequence of the diabetic disorder or just an accompanying symptom, remains to be clarified (German). M.G.G.

Davis, T. W.; Kerr, R. B.; and Bogoch, A. (Dept. of Med., Shaughnessy Hosp. & Univ. of Brit. Col., Vancouver, B.C., Canada): EXPERIENCE WITH GLIPASOL (R.P. 2259)—AN ANTIDIABETIC SULFONAMIDE DRUG. Canad. M. A. J. *81*:101-07, July 15, 1959.

This is a report on the use of a new "antidiabetic" sulfonamide. It has an amine group on the benzene ring, as has carbutamide, but a thiodiazole is substituted for the urea nucleus. Thirty-one patients with "maturity-onset" of diabetes were treated with doses varying from 1.0 to 3.0 gm. per day. Effectiveness and therapeutic results were similar to other sul-

fonylureas. One patient who was showing poor responses to the drug died of cardiac disease. Autopsy showed a paucity of pancreatic insulin.

The toxic effects of the drug included leukopenia and hepatic necrosis. Six of the thirty-one patients developed jaundice. Liver biopsies in five showed diffuse parenchymal degeneration. Eleven other nonjaundiced patients showed laboratory evidence of hepatic dysfunction. It was concluded that the drug was too toxic for clinical use. S.B.B.

Dodge, Philip R.; Mancall, Elliott L.; Crawford, John D.; Knapp, John; and Paine, Richmond S. (Harvard Med. Sch., Mass. Gen. Hosp., and Childrens' Med. Center, Boston, Mass.): HYPOGLYCEMIA COMPLICATING TREATMENT OF PHENYLKETONURIA WITH A PHENYLALANINE-DEFICIENT DIET. New England J. Med. 260:1104-11, May 28, 1959.

Two phenylketonuric children in whom hypoglycemia developed during the course of treatment with phenylalanine-restricted diet are described and hypoglycemia is considered a complication of the treatment because of refusal to take an adequate amount of an unpalatable diet over a period of weeks or months resulting in a state of undernutrition accompanied by fatty metamorphosis of the liver. Neuropathological findings were similar to those seen after prolonged hypoglycemia. S.S.

Drey, Norman W.; Taussig, Barrett L.; and Rubin, Robert (Jewish Hosp. and Dept. of Med., St. Louis Univ. Sch. of Med., St. Louis, Mo.): ORAL HYPOGLYCEMIC SULFONYLUREA DRUGS: FOUR YEARS' EXPERIENCE IN THE TREATMENT OF ADULT DIABETES MELLITUS. Missouri Med. 56:1015-19, September 1959.

The authors review their experience in the treatment of diabetes with oral hypoglycemic agents over the past four years. Drugs used were tolbutamide, chlorpropamide and metahexamide. Tolbutamide and chlorpropamide were found to be useful in adult-type diabetics, but are not recommended for juvenile diabetics or obese adult diabetics who can be controlled by weight reduction and diet. Metahexamide gave a good hypoglycemic effect but signs of liver toxicity were observed in five of six patients and the drug is no longer being used. R.L.J.

Farquhar, James W. (Dept. of Child Life and Health, Univ. of Edinburgh, and the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh, Scotland): THE CHILD OF THE DIABETIC WOMAN. Arch. Dis. Childhood 34:76-96, February 1959.

Although the advent of insulin improved the fertility of diabetic women, it did not solve the problem of the diabetic pregnancy in which the fetal loss is high. The fetus may die in utero or in the first few days of life. The babies are heavy and have dyspnea and cyanosis. At autopsy, pulmonary hyaline membrane and hyperplasia of the pancreatic islets are frequently found. The incidence of congenital malformations may be no higher. Height and weight after infancy are average. R.L.J.

Field, James B. (Nat'l. Inst. of Arth. and Metab. Diseases, Nat'l. Inst. of Health, Bethesda, Md.): ON THE NATURE OF THE METABOLIC DEFECT(S) IN DIABETES. Am. J. Med. 26:662-71, May 1959.

The author gives a clear, concise, current review of the present concepts of the metabolic defects in diabetics indicating that the metabolic abnormality is both complex and incompletely understood, and that animal experimentation, though having yielded much pertinent information, fails to match adequately the various clinical types of diabetes. He further indicates that the various types of clinical diabetes cannot as yet be explained on the same etiologic or biochemical defects. Included among the unanswered problems of clinical diabetes are the marked difference in insulin production by the pancreas of the juvenile and adult diabetic patients, the question of direct insulin effect upon hepatic carbohydrate metabolism, the exact mechanism by which hyperglycemias are produced whether this be under-utilization or overproduction of glucose, the apparent discrepancy in the rate of glucose oxidation to carbon dioxide in the juvenile as compared to the obese diabetic, the exact mechanism responsible for impairment of the diabetic subject in the synthesis of fatty acids, the defect in protein synthesis and in the Krebs cycle. L.S.S.

Foa, Piero P.; and Galansino, Giorgio (Dept. of Physiol. and Pharmacol., The Chicago Med. Sch., National Inst. of Arthritis and Metabolic Diseases, Public Health Serv., Chicago, Ill.): THE ORALLY ACTIVE HYPOGLYCEMIC AGENTS. Chicago M. Sch. Quart. 20:80-87, June 1959.

The experimental evidence reviewed in this paper suggests that sulfonylureas act by suppressing liver glucose production and that this action is possible only when insulin is injected or released in "permissive" amounts. This hypothesis would explain why the drugs cause hypoglycemia in the absence of the liver when glycogenolysis is absent, insulin secretion normal and insulin destruction normal or reduced, and why they are not effective in the absence of functioning pancreatic tissue or of exogenous insulin when glycogenolysis and gluconeogenesis proceed at an overwhelming rate. Thus, carbutamide and tolbutamide may exert their hypoglycemic effect only in normal, partially alloxanized or partially depancreatized animals, or in mild diabetic patients where insulin may be provided by the stimulation of the β-cells in quantities sufficient to make the liver action of the drugs possible. If this hypothesis is correct, prolonged therapy with sulfonylurea-like drugs should be attempted with caution, for the suppression of hepatic glucose production may be a sign of injury and, although continued stimulation of the β-cells may result in hypertrophy and increased insulin production, it may lead also to functional insufficiency. In addition to the possibility of functional and structural damage, harm may be done by withholding insulin therapy; the problem of diabetic complications has not been solved, as a disturbingly large number of patients still suffer from neuritis, retinitis, lens opacities, glomerular and other vascular lesions. For these reasons it may be wise to give the patients the benefit of the doubt and use oral therapy only as an adjuvant to insulin or in those patients who cannot be treated with diet alone, who refuse insulin or for whom the use of insulin is made hazardous by physical or mental handicaps. H.L.W.

Granville-Grossman, K. L.; Crawfurd, S.; Crowley, Mory F.; and Bloom, Arnold (Whittington Hosp., London, England): FURTHER EXPERIENCE WITH ORAL THERAPY IN DIABETES. Brit. M. J. 2:841-47, Oct. 31, 1959.

Metahexamide (thirty patients) is a well-tolerated hypoglycemic agent, effective in mild diabetics with onset at maturity. Its action is more sustained than that of tolbutamide, so it can be administered once daily. Jaundice was observed in one patient after treatment with the drug.

Chlorpropamide (seventy-five patients) is a more effective oral hypoglycemic agent than tolbutamide, and need be given only once a day. It is well tolerated, but occasionally gives rise to sensitivity reactions.

Dimethyldiguanide (eight patients) is an effective hypoglycemic agent but is not well tolerated by mouth.

Half-life studies on metahexamide show that the drug disappears from the plasma more rapidly in normal individuals than in diabetic patients.

Many diabetics, particularly new ones, remain controlled by a simple dietary restriction alone after a six-weeks' course of oral therapy, though the length of remission is variable.

Three diabetic patients who failed to respond either to a sulfonylurea derivative or to phenethyldiguanide when administered separately were stabilized on a combination of the two. J.A.

Greenberg, Stephen R. (The Chicago Med. Sch., Chicago, Ill.): EXPERIMENTAL DIABETES: A HISTORY OF BASIC RESEARCH. Chicago M. Sch. Quart. 20:88-94, June 1959.

The author traces the development of basic research in diabetes mellitus and shows how the contributions which have been forthcoming are singularly and mutually effective in advancing the understanding of this disease. W.R.K.

Hagen, Jean M.; Ball, Eric G.; and Cooper, Octavia (Dept. of Biol. Chem., Harvard Med. Sch., Boston, Mass.): STUDIES ON THE METABOLISM OF ADIPOSE TISSUE. II. THE EFFECT OF CHANGES IN THE IONIC COMPOSITION OF THE MEDIUM UPON THE RESPONSE TO INSULIN. J. Biol. Chem. *234*:781-86, April 1959.

The effect of variations in the site of sampling, in changes in the medium and in the age of the animals on the accuracy of the rat epididymal fat method of insulin assay was determined. There was good agreement between activity of four different portions of fat taken from the same animal. Older rats exhibited a decreased response of the epididymal fat pad to insulin. It is recommended that the Krebs-Ringer bicarbonate solution be used for the medium, and that gelatin should be added to the medium when physiological doses of insulin are employed. A.R.C., JR.

Hausberger, Franz X.; and Ramsay, Andrew J. (Jefferson Med. Coll. of Philadelphia, Pa.): ISLET HYPERTROPHY IN OBESITY OF MICE BEARING ACTH-SECRETING TUMORS. Endocrinology 65:165-71, August 1959.

Grafts of ACTH secreting anterior pituitary tumors caused the recipient mice to gain weight due to excessive deposition of adipose tisuue. An increase in size and number of pancreatic beta cells was seen in the obese mice. Beta cell degranulation paralleled the degree of obesity. Islet hypertrophy still develops in spite of food restriction. Force-fed normal rats show no islet hypertrophy. These findings and the fact that insulin speeds adipose tissue lipogenesis suggest that increased secretion of insulin is responsible for obesity in this form of hyperadrenocorticism. H.L.W.

Hill, John B. (Dept. of Pharmacol., Univ. of North Carolina, Chapel Hill, N. C.): THE ADSORPTION OF I^{131}-INSULIN TO GLASS. Endocrinology 65:515-17, September 1959.

I^{131}-insulin in acidified saline solution is adsorbed to the walls of glass containers in amounts which would significantly alter micro-insulin assays. The addition of gelatin (5 per cent solution) prevents loss of insulin by adsorption. I^{131}-insulin which has been permitted to adsorb to the glass walls of containers will elute in protein solutions subsequently placed in the containers and in this fashion may provide a source of contamination. H.L.W.

Ito, Tomiko (Dept. of Pediat., The New York Hosp., Cornell Med. Center, New York, N. Y.): MATERNAL DIABETES MELLITUS AND THE NEWBORN INFANT. Quart. Rev. Pediat. *14*: 176-81, July-Sept. 1959.

In well controlled diabetes in the absence of overt vascular disease, pregnancy is possible without added risk to the mother. The perinatal mortality, however, continues to be high. The author discusses infants born to diabetic mothers from the standpoints of pathology, neonatal complications, and treatment. R.L.J.

Janes, Ralph G.; Alert, Harold A.; and Johnson, Nadine K. (Dept. of Anat., State Univ. of Iowa Coll. of Med., Iowa City, Ia.): DEPENDENCE OF AQUEOUS-BLOOD SUGAR RATIOS ON METHOD OF DETERMINATION. A. M. A. Arch. Ophth. *61*: 720-26, May 1959.

In estimations of glucose in rabbit blood and aqueous humor, the Somogyi-Nelson and glucose oxidase methods were found to be the most accurate of several technics and to agree closely with each other. A.R.C., JR.

Joplin, G. F.; Fraser, Russell; and Vallance-Owen, J. (Postgraduate Med. Sch. of London, W. 12; Dept. of Med., King's College, Newcastle upon Tyne, 1, England): TOLBUTAMIDE CONTROL OF DIABETES MELLITUS: SELECTION OF PATIENTS AND PERSISTENCE OF RESPONSE. Lancet 2:582-84, Oct. 17, 1959.

A method is presented for the selection of diabetic patients suitable for control by tolbutamide. Response to treatment may be assessed by the determination of one fasting sugar in addition to twenty-one home urine tests performed during two-week periods on placebo and on tolbutamide. Ketosis is a clear contraindication to tolbutamide. With mild diabetes not controlled by diet there is no simple clinical criteria to determine which will respond favorably to tolbutamide. A fasting blood sugar of over 250 mg. per cent suggests that the patient will not respond to sulfonylurea treatment.

Throughout a year of therapy on tolbutamide the same response was maintained in twenty-one out of twenty-four "good responders" and in eight out of nine "fair responders." Failure of the drug was little influenced by change in dosage. J.A.

Kalk, H. (Stadtkrankenhaus, Medizinische Abteil, Kassel, Germany): THE RELATIONSHIP BETWEEN FATTY LIVER AND DIABETES MELLITUS. Deutsche med. Wchnschr. *84*:1898-1901, Oct. 16, 1959.

Liver biopsies in 121 diabetic patients revealed fatty livers in forty-nine cases. In all instances the diabetes was of maturity onset and the patients were older than forty years. No data on the nutritional status of the patients are given. The fatty liver of diabetes was characterized histologically by coarse fat droplets and prominent vacuolization, lighter color and variation in size of the liver cell nuclei. A similar picture was observed also in the livers of patients whose diabetes became manifest only some time after the biopsy. This suggests that there is a prediabetic stage which consists of a still compensated disorder of carbohydrate metabolism, but nevertheless already noticeable changes in fat metabolism. While the development of diabetes could be predicted from the character-

istic histological picture in the liver, it was also possible to induce a regression of the fatty changes and to prevent the manifestation of the diabetes by placing such patients on low caloric, low carbohydrate and nonalcoholic diets. Cirrhosis of the liver was found in thirty-nine of the 121 patients. This incidence is considerably greater than generally assumed. This may be due in part to selection of the material. In about two thirds of these cases, the diabetes preceded the cirrhosis. In the sixteen cases where hepatitis and subsquent cirrhosis was the primary disease, it is assumed that the pancreas was involved secondarily causing islet cell degeneration. M.G.G.

Lee, Norman D. (Univ. of Tenn. Medical School, Memphis, Tenn.): THE SPECIFICITY OF THE INTERACTION OF INSULIN OF INSULIN-I^{131} WITH TISSUE. Endocrinology *63*:347-56, September 1959.

Radio-iodine labeled insulin retaining the ability to lower blood sugar exhibits a characteristic distribution and fate in the rat. Radio-iodine labeled insulin treated with sodium hydroxide or with an aqueous solution of iodine loses its ability to lower blood sugar and shows: (1) a reduced concentration of radioactivity in the liver and kidney, (2) less firm binding to liver and (3) different distribution of radioactivity to intracellular structures as compared with the intact labeled hormone. Rate of degradation is different for the intact hormone, the sodium hydroxide inactivated hormone and the iodine inactivated hormone.

Iodine labeling of insulin as commonly employed does not result in a loss of potency. Only when iodine substitution exceeds two iodine atoms per molecule does inactivation occur. It seems reasonable to believe that iodination of insulin within set limits does not appreciably alter the hormone. H.L.W.

Lee, Norman D.; and Wiseman, Ralph, Jr. (Radioisotope Serv., V. A. Med. Teaching Group Hosp., and the Dept. of Chemistry, Univ. of Tenn. Med. Sch., Memphis, Tenn.): A SIMPLIFIED AND EXACT METHOD FOR THE CONTROLLED SUBSTITUTION OF PROTEINS WITH I^{131}. J. Lab. & Clin. Med. *54*: 325-32, August 1959.

This report presents a simple, easy, and rapid method for preparing pure aqueous I_2-I^{131} solutions from KI-KIO_3 and carrier-free I^{131}. This method permits the exact determination and control of I_2 concentration and I^{131} specific activity. Data are presented showing the use of such solutions in the iodination of insulin. G.J.H.

Lee, Norman D.; and Wiseman, Ralph, Jr. (Univ. of Tenn. Med. Sch., Memphis, Tenn.): THE SIGNIFICANCE OF THE BINDING OF INSULIN I^{131} TO CYTOSTRUCTURAL ELEMENTS OF RAT LIVER. Endocrinology *65*:442-50, September 1959.

Distribution of intravenously administered tracer doses of insulin I^{131} was given study. The intracellular insulin I^{131} was localized largely in mitochondria and microsomes. Insulin bound to these structures in the intact cells was more firmly fixed than insulin added to these structures isolated from cells. Binding to mitochondria and microsomes provided a degree of protection to the hormone from endogenous degradative processes and from insulinase. H.L.W.

MacDonald, Mary K. (Pathology Dept., Univ. of Edinburgh, Scotland): HISTOCHEMICAL OBSERVATIONS ON THE SULPHYRDYL CONTENT OF PANCREATIC ISLETS AFTER ADMINISTRATION OF DIABETOGENIC SUBSTANCES. Quart. J. Exper. Physiol. 44:177-82, April 1959.

Lazarow (*Physiol. Rev.* 29:48-74, 1949), has proposed that substances such as alloxan and dehydroascorbic acid (DHA) produce diabetes by destroying free sulphydryl groups in the body. Because such groups exist normally at a critical level in the beta cells, these cells may succumb to such treatment. A histologic method of tissue treatment has been applied whereby the free sulphydryl groups present as glutathione are largely preserved, and groups bound in tissue proteins are not liberated by denaturation. The pancreases of rats killed at varying intervals after injection with alloxan or with DHA, and of control rats, have been treated in this way and subsequently stained by Benett's red sulphydryl agent. No apparent difference was seen in the sulphydryl content of normal acinar and islet tissue. After injection of alloxan or DHA a reduction of sulphydryl groups in the beta cells was regularly observed, results which are consistent with Lazarow's main hypothesis. G.A.W.

Marks, I. N.; Shuman, Charles R.; and Shay, Harry (Fels Res. Inst. & Diabetic Serv., Temple Univ. Med. Center, Philadelphia, Pa.): GASTRIC ACID SECRETION IN DIABETES MELLITUS. Ann. Int. Med. *51*:227-37, August 1959.

An investigation of the acid secretion of gastric mucosa of forty-one diabetic patients. No quantitative difference was found among these patients as compared with the normal. S.B.B.

Meyer, W. H., Jr.; and Starzl, T. E. (Dept. of Surgery, Univ. of Miami Sch. of Med. and Jackson Mem. Hosp., Miami, Fla.): THE EFFECT OF ECK AND REVERSE ECK FISTULA IN DOGS WITH EXPERIMENTAL DIABETES MELLITUS. Surgery *45*:760-64, May 1959.

Construction of Eck fistulas resulted in an increased severity of alloxan diabetes in five dogs. This was thought to be caused by impairment in liver function or by deviation of alimentary glucose away from control by the liver. Increasing hepatic blood flow by creating a reverse Eck fistula failed to affect the diabetes. Diabetic dogs with Eck fistulas survived longer than nondiabetic operated animals. A.R.C., JR.

Mitchell, Marvin L.; Whitehead, Wm. O.; and O'Rourke, Mary E. (Lemuel Shattuck Hosp. and Tufts Univ. Sch. of Medicine, Boston, Mass.): DIFFERENTIAL RESIN BINDING OF INSULIN IN SERUM. Endocrinology *65*:322-33, August 1959.

A method has been devised which facilitates the study of insulin binding by serum proteins. A strongly basic anion exchange resin which competes with serum proteins for iodine-labeled insulin is employed. It was demonstrated that the sera from insulin-treated diabetic patients bound labeled insulin to a greater extent than sera from normal individuals. The binding of insulin to the resin was affected by competition between serum proteins and insulin for the available sites of adsorption on the resin and also by the maximum adsorbing capacity of the resin. There was no correlation between insulin binding by serum and insulin requirement in the patients studied. No insulin resistant patients were included. H.L.W.

Moore, Edward W.; Mitchell, Marvin L.; Chalmers, Thomas C. (Med. Services & Radioisotope Unit, Lemuel Shattuck Hosp., Harvard & Tufts Med. Schools, Boston, Mass.): VARIABILITY IN ABSORPTION OF INSULIN-I^{131} IN NORMAL AND DIABETIC SUBJECTS AFTER SUBCUTANEOUS AND INTRAMUSCULAR INJECTION. J. Clin. Invest. *38*:1222-27, July 1959.

A study of the rate of absorption of I^{131} tagged insulin given both subcutaneously and intramuscularly to diabetic and

nondiabetic humans. The greater the volume of solution the greater was the speed of absorption. There was considerable variation in absorption time, namely, one to six hours. There was no essential difference between subcutaneous and intramuscular sites, but variations were considerable from patient to patient, or in the same patient at different times and sites, two diabetics, showing sixteen- to twenty-four-hour absorption times. Diabetics showed increase in absorption times as compared with normals only by the intramuscular route. The authors inferred that this indicated some form of tissue binding of insulin in diabetics. S.B.B.

Moss, James M.; DeLawter, DeWitt, E.; and Canary, John J. (Diabetic Clinic, Georgetown Univ. Hosp., Washington, D.C.): RESULTS OF THE TREATMENT WITH TOLBUTAMIDE OF 200 DIABETIC PATIENTS: A DISCUSSION OF SECONDARY FAILURE. Ann. Int. Med. 50:1407-17, June 1959.

The authors detailed their experience with tolbutamide therapy of two hundred adult diabetics. They analyzed their results with respect to gradation of initial success and secondary failure. Primary failure occurred in a total of 17 per cent and secondary failure in 16 per cent of the patients. The latter occurred after varying degrees of initial success and to an equal extent in the first and second six months of therapy.

The use of placebo controlled experiments revealed the role of suggestion in therapy of the milder, obese diabetic. Tolbutamide was continued successfully during intercurrent surgery and pregnancy. Insulin requirement was not increased following secondary tolbutamide failure. S.B.B.

Perdue, George W.; Engelhardt, Hugo T.; and Coronado, Marguerite (Baylor University Coll. of Med., and Veterans Administration Hosp., Houston, Tex.): DIETS TO CONTROL DIABETES MELLITUS: EFFECT OF MODIFICATION TO MEET DIETARY HABITS OF INDIGENT ANGLO-, NEGRO-, AND LATIN-AMERICAN PATIENTS. Texas J. Med. 55:283-86, April 1959.

Diabetic diets of one hundred outpatients in a large hospital for indigents were modified to fit into their food habits and capability to pay for the foods. The diets were modified into three types, Anglo-American, Negro-American, and Latin-American. In forty-six instances, the diet modification apparently permitted the patient to adhere more strictly to the prescribed diet. Modification of the diet did not alter the control of the diabetes in forty-two patients. Control of the diabetes was less satisfactory in twelve patients, but this was not due to the modification of the diet. The financial status and food habits of the patient should be taken into consideration in the diet prescription and menus given to diabetic patients. B.F.K.

Pomeranze, Julius (New York Medical College, Metropolitan Medical Center, New York, N.Y.): SUBTHRESHOLD DIABETES. Ann. Int. Med. 51:219-26, August 1959.

An analysis of the symptoms and complications occurring among 1,140 adult diabetics at or before diagnosis of the disease. There was a positive family history in 61 per cent and obesity in 83 per cent. Hypoglycemic symptoms were elicited in 111 of these patients before diagnosis and also among 110 mild diabetics after diagnosis. Complications such as neuropathy, cataract, impotence, and diabetic diarrhea were noted prior to diagnosis of the disease to a significant degree. S.B.B.

Pomeranze, Julius; Stone, Martin L.; and King, Edward J. (Maternal and Newborn Res. Lab., Dept. of Obstetrics and Gynecology, Dept. of Med., New York Med. Coll., Metropolitan Med. Center, New York, N.Y.): THE OBSTETRIC IMPORTANCE OF OBESITY AND "BENIGN" GLYCOSURIA IN PREDIAGNOSIS DIABETES. Obst. & Gynec. 13:181-84, February 1959.

"Benign glycosuria" is not benign from the prognostic point of view. The presence of glycosuria, even with normoglycemia, should be considered with great care for its effect on the fetus, and as an indicator of the future development of diabetes mellitus. Control of diabetes itself during pregnancy is satisfactorily accomplished in most cases. But good diabetic control may not significantly alter the fetal or neonatal mortality or the increased size of the fetus. It is perhaps then too late and the metabolic reserve is too limited to respond to the alleviation of stress by good clinical control of the diabetes. In the prediagnosis era, reserve is still good and the elimination of precipitating stress such as obesity may control the complications of the pregnancy as well as delay the development of the clinically evident syndrome. There seems little doubt that obesity increases the hazards of childbearing. The high incidence of diabetes in married women appeared to be related, at least in part, to previous childbearing and the presence of obesity. The vigorous application of therapeutic efforts especially in the establishment and maintenance of good nutrition and normal weight could have prevented or delayed the appearance of diabetes mellitus and accelerated degeneration and embryopathy. A metabolic insufficiency is stressed sufficiently by obesity, an unknown stress factor of pregnancy, other extraneous factors, and the aging process. Accelerated degenerative changes associated with this inadequacy and the fatal fetal factor are already present and lead to the known mischances of pregnancy and the earlier unveiling of diabetes mellitus. W.R.K.

Rakel, Robert E.; Skillman, Thomas G.; and Braunstein, John R. (Sr. Med. Student, College of Med., Univ. of Cincinnati; Asst. Prof. of Med., Div. of Metabolism & Endocrinology, Ohio State Univ., Columbus; Assoc. Prof. of Biophysics, Univ. of Cincinnati; Cincinnati Gen. Hosp., Ohio): THE BALLISTOCARDIOGRAM IN JUVENILE DIABETES MELLITUS. Circulation 18:637-39, Part 1, October 1958.

The authors studied forty-one patients with juvenile diabetes, all of whom had had onset of diabetes prior to the age of fifteen, with ballistocardiogram tracings. None of these patients had any evidence of cardiac disease by history, physical examination, X-ray, or electrocardiographic studies.

Of the forty-one patients, seventeen, or 41 per cent, had abnormal ballistocardiographic patterns. In a control group no irregularities were found. No single pattern of the ballistocardiogram seemed to predominate. From these findings it is suggested that the abnormalities were related to early development of coronary sclerosis. R.C.C.

Ross, Eric J.; Crabbe, Jean; Renold, Albert; Emerson, Kendall, Jr.; and Thorn, George W. (Peter Bent Brigham Hosp., Dept. of Med., Harvard Med. Sch., Boston, Mass.): A CASE OF MASSIVE EDEMA IN ASSOCIATION WITH AN ALDOSTERONE-SECRETING ADRENOCORTICAL ADENOMA. Am. J. Med. 25: 278-92, August 1958.

A very detailed account of this most unusual case is presented. The patient had marked edema, ascites, and pleural

effusion. She was unresponsive to numerous diuretic procedures including dialysis on the artificial kidney. Prompt weight loss occurred following the removal of the left adrenal which contained an adenoma but weight gain and fluid retention recurred and were again improved by removal of the right adrenal which demonstrated hypertrophy. It was felt that the massive edema resulted from a defect of capillary permeability resulting in a secondary hyperaldosteronism. Freedom from edema has not been achieved due to the appearance of symptoms suggestive of sodium depletion when her weight falls below a certain level. R.W.S.

Rubini, Milton E.; and Seligson, David (Dept. of Metabolism, Div. of Med., Walter Reed Army Inst. of Res., Washington, D.C.): THE ACUTE REDUCTION IN PLASMA AMINO ACIDS BY CARBOHYDRATE INFUSION IN DIABETES AND LIVER DISEASE. Am. J. Clin. Nutrition 6:365-75, July-August 1958.

The plasma amino acid nitrogen in fasting normal animals and man is reduced by exercise, insulin and glucose feeding. It is theorized that this decrease in plasma amino acid is due to either increase in protein synthesis or decrease in protein breakdown. The study by the authors confirmed previous findings of reduction in normal men by increasing normal carbohydrate utilization. They also substituted fructose and included a group of diabetic patients and presented evidence that this phenomenon of plasma amino acid reduction is independent to a large degree of the insulin reaction. Also, the fall in plasma amino acids did not appear to have any direct relationship to the blood sugar level other than to the increased rate of carbohydrate utilization. Plasma amino acids were also studied in a group of patients with liver disease with carbohydrate infusion, and in dogs with choline deficiency and fatty metamorphosis of the liver, and approximately half of these patients with liver disease responded abnormally. The authors feel that this indicates that intact hepatocellular function is necessary for the reduction of amino acids following carbohydrate infusion and they suggest that the liver is the major site of removal of amino acids from the plasma under these circumstances. R.C.C.

Schliack, Volker (Diabetes-Zentrale, Berlin C2, Kloster str. 64, Karlsburg, Germany): SERIAL EXAMINATIONS FOR DIABETES MELLITUS (DIARETES REIHENUNTERSUCHUNGEN). Deutsche med. Wchnschr. *84*:1446-48, Aug. 14, 1959.

The author and his associates have carried out diabetes detection drives in various parts of Germany and attempted to survey representative population groups both from rural and metropolitan areas, from Eastern and Western Germany. Their report is based on a study of 16,722 people. The over-all incidence of diabetes in Germany appeared to be 1.5 per cent. There was little geographic difference in incidence or between rural and industrialized areas. Related to age, the incidence was highest above forty (3-5 per cent) and higher among women than among men. The discovery of previously unknown diabetes was insignificantly small in the age group below forty. Among the people above forty years of age the number of newly detected diabetics equalled or even surpassed the number of known diabetics. The newly detected diabetes was usually mild and controllable by diet alone, but a significantly high incidence of angiopathies and other diabetic complications was discovered in this group. M.G.G.

Sellman, J. C.; Perkoff, G. T.; Null, F. C.; Kimmel, J. R.; and Tyler, F. H. (Univ. Utah Coll. Med., Salt Lake City, Utah): HYPOGLYCEMIA ASSOCIATED WITH MASSIVE INTRA-ABDOMINAL MESOTHELIAL-CELL SARCOMA. New England J. Med. 260:847-52, April 23, 1959.

A case of massive intra-abdominal mesothelial-cell sarcoma with associated hypoglycemia is reported. Insulin-like activity could not be demonstrated in extracts from the tumor. The tumor was found to have high levels of amino acids and glycogen. The high glycogen and amino acid levels together with the absence of demonstrable evidence of other mechanisms for the production of hypoglycemia, suggests that excessive glucose consumption by the tumor was responsible for the hypoglycemia in this patient. S.S.

Sloan, Norman R. (Bureau of Geriatrics, Hawaii Dept. of Health, Honolulu, Hawaii): DIABETES IN HAWAII: PART I: PREVALENCE. Hawaii M. J. *18*:485-87, May-June 1959.

There are 6,000 residents of Hawaii who have diabetes mellitus, and half of these are as yet undiagnosed and untreated. This is true if the statistics so far obtained from the Health Department's current survey can be applied to Hawaii as a whole. A study of deaths reported as due to diabetes, in the years 1949-54, shows age-adjusted rates of 56.4 per 100,000 for Hawaiian and part Hawaiian, 19.1 for Japanese, 13.1 for Filipino. The figure 36.1 for Caucasian has no value here, as it includes Portuguese, an epidemiologically distinct group. A study by Berk, Page, and Herbst on 232 patients in The Queen's Hospital diabetes clinic, 1940-45, showed a relative preponderance of Hawaiian and part-Hawaiian patients, and a very low rate among Japanese, as compared with the total numbers of these groups in the general population. A project now under way has a twofold purpose: first, to locate undiagnosed cases of diabetes mellitus and second, to determine if there are significant differences in the ethnic distribution of diabetes among the people of Hawaii. During the fourteen months to Feb. 28, 1959, 24,077 participants were screened. Of the known diabetics who participated in the survey, 145 have been verified as previously known to their attending physicians and 152 were reported as newly diagnosed cases. If the figures are applied to the current population estimate of 585,000 for Hawaii, a figure of 7,216 is obtained, indicating that the opening sentence is conservative. W.R.K.

Vallance-Owen, J.; Joplin, G. F.; and Fraser, Russell (Dept. of Medicine, King's College, Newcastle upon Tyne, 1; Postgraduate Med. Sch. of London, W.12, England): TOLBUTAMIDE CONTROL OF DIABETES MELLITUS: CLINICAL RESPONSIVENESS AND INSULIN RESERVE. Lancet 2:584-86, Oct. 17, 1959.

The blood sugar and plasma-insulin activity before and two and one-half hours after tolbutamide (2 gm.) was measured in five normal and twenty-two diabetic subjects. In this acute test the plasma insulin activity rose in both normal and clinically responsive diabetics. No response was observed in the clinically nonresponsive group. The fall in blood sugar correlated with the rise in plasma-insulin activity. Insulin activity was assayed by the increase in sugar uptake of the isolated rat diaphragm.

The authors conclude that tolbutamide lowers blood sugar by stimulating endogenous insulin production when there is an adequate pancreatic insulin reserve. J.A.

ORGANIZATION SECTION

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20TH ANNUAL MEETING

The American Diabetes Association will hold its Twentieth Annual Meeting in Miami Beach, Florida, June 11-12, 1960, at the Deauville Hotel, prior to the annual session of the American Medical Association. Additional information about the Annual Meeting together with a hotel reservation card was mailed to members of the Association in November. *Please complete your card and send it directly to the Deauville Hotel as soon as possible.*

The Annual Banquet and Social Hour will be held Saturday evening, June 11. Members, their wives (or husbands) and friends are cordially invited to attend both the Banquet and Social Hour. A reservation form for the Banquet will be mailed a few months prior to the meeting.

If you are planning to attend the ADA Twentieth Annual Meeting and to stay over for the annual meeting of the American Medical Association, which follows on June 13-17, inform the Deauville Hotel that you would like to continue your ADA reservation for the AMA sessions.

SCIENTIFIC PROGRAM

Priscilla White, M.D., of the Joslin Clinic and the New England Deaconess Hospital, Boston, Massachusetts, will deliver the Banting Memorial Lecture. Her subject will be on juvenile diabetes.

Physicians and other scientists who would like to present papers at the Scientific Sessions are invited to submit abstracts to Blair Holcomb, M.D., Chairman of the Committee on Scientific Programs. These sessions will be held on Saturday and Sunday, June 11-12. Seven copies of the abstracts, which are requested in order to expedite review by the Committee, should be transmitted as soon as possible, but not later than March 1, 1960, to Dr. Holcomb in care of the national office.

A list of papers to be given at the Scientific Sessions will be published in DIABETES, and the program itself, including abstracts of all the papers, will be sent out to members before the meeting.

8TH GRADUATE AND MEDICAL STUDENT-INTERN ESSAY CONTEST

Members of the American Diabetes Association and subscribers to DIABETES are asked to encourage graduate students, medical students, interns and physicians within two years after their graduation from medical school to enter the 1959-60 Graduate and Medical Student-Intern Essay Contest.

An award of $250 will be given to the author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation. One hundred dollars ($100) will be awarded for the best review article or case report.

The papers will be judged on the basis of the value of the material and method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Contestants should submit the original and two copies of

their papers. Typewritten and double-spaced, all manuscripts should be mailed by April 1, 1960, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y.

FOURTH CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

The Fourth Congress of the International Diabetes Federation will be held in Geneva, Switzerland, July 10-14, 1961. Other pertinent information will be published in the Journal as soon as it is received.

PROGRAM DIRECTORS' WORKSHOP

The American Diabetes Association will co-sponsor with the National Institute of Arthritis and Metabolic Diseases a "Diabetes Program Directors' Workshop" on Friday and Saturday Jan. 22-23, 1960, immediately following the Eighth Postgraduate Course Jan. 20-22, 1960, at the Ambassador Hotel in Los Angeles. Arthur R. Colwell, Sr., M.D., will be Director of the Workshop. The meeting will provide an opportunity for Program Directors of NIAMD Diabetes Training Grants to exchange information and experiences. Your Postgraduate Course badge will admit you to these open sessions: January 22, 8-10 p.m.—General Session, Embassy Room; January 23, 4-7 p.m. —Plenary Session, Grove Lounge.

PROCEEDINGS OF CONFERENCE ON TEACHING AND RESEARCH IN DIABETES

The proceedings of the first National Conference on Teaching and Research in Diabetes are available without cost by addressing your request to American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y. Entitled *Teaching and Research in Diabetes,* the proceedings were edited by E. Perry McCullagh, M.D. The Conference Committee included E. Perry McCullagh, M.D., Chairman, Arthur R. Colwell, Sr., M.D., John E. Howard, M.D., Thomas McGavack, M.D., and Robert H. Williams, M.D.

Copies already have been sent to all members of the American Diabetes Association, representatives who attended the Conference, Deans of Medical Schools, Professors of Medicine, Diabetes and Metabolism Program Directors, all member organizations of the International Diabetes Federation, and other interested persons.

The first National Conference was held at Chalfonte-Haddon Hall, Atlantic City, New Jersey, May 3, 1958, under the sponsorship of the American Diabetes Association and the National Institute of Arthritis and Metabolic Diseases, Public Health Service, U. S. Department of Health, Education and Welfare.

ORAL COMPOUNDS ARTICLE IN ADA *FORECAST*

An up-to-the-minute report on the oral hypoglycemic compounds, entitled "Current Status of Oral Compounds for Diabetes," by Rachmiel Levine, M.D., Chairman, Informational Committee on Oral Hypoglycemic Compounds, American Diabetes Association, appears in the Jan.-Feb. 1960 issue of ADA FORECAST. It is concerned with the history of the development of these compounds and reports their clinical usefulness. Structural formulae of tolbutamide, chlorpropamide and phenformin are given. Single copies of the FORECAST are available on request at 35¢ each, or six for $2.00. Reprints of the article may be obtained at 10¢ each or three for 25¢. Quantity orders of both the FORECAST and reprints are available at substantially reduced prices.

DIABETES WEEK LARGER THAN EVER

The most productive Diabetes Week in ADA history was launched on November 15 when a telegram was received from President Eisenhower which read as follows:

"The Diabetes Detection Campaign sponsored by the American Diabetes Association offers our citizens the opportunity for early diagnosis leading to successful treatment. I wish the Association every success in its continuing efforts to reduce the toll of this disease."

At the conclusion of Diabetes Week on November 21, it appeared that a larger number of persons was tested than in previous years, and that the educational program reached more people than ever before.

DIABETES BINDERS AVAILABLE

Sturdy binders for your 1960 issues of DIABETES are now available at a price of $2.25 each. For those readers who need binders for prior years, these also are available at the same price.

A full set of binders for all nine volumes (1952-1960 issues) is offered at a package price of $18.00.

Each of these attractive binders will hold a year's issues of DIABETES and also the annual index supplement. Of simulated dark blue leather, they will grace your library shelf and add years of life to your copies of DIABETES. Address your orders now for immediate shipment to: American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y.

NEW MEMBERS

The following were elected as of Dec. 1, 1959, and Jan. 1, 1960.

Active

California	
Bailey, Wm. T., Jr.	San Diego
Loranger, Robert H.	Pasadena
Georgia	
Eyzaguirre, Guillermo	Atlanta
Indiana	
Miller, Hugh A., Jr.	Elkhart
Louisiana	
Gladney, James F., Jr.	Homer
Maryland	
Matchar, Joseph C.	Baltimore
Shaw, Charles E., Jr.	Baltimore

Michigan	
Litwin, Jack A.	Detroit
Upjohn, Harold L.	Kalamazoo
Minnesota	
Hoffman, David L.	Rochester
New Hampshire	
James, Richard H.	Keene
Jessup, James S.	Laconia
Vanderlinde, Robert J.	Hanover
New York	
Casey, Thomas V.	Troy
Pedlow, Harry J.	Pleasantville
Shreeve, Walton W.	Upton
Spitz, William C.	Brooklyn
Nevada	
Belcourt, Claude M.	Reno
Pennsylvania	
McMorris, David L.	Williamsport
Saras, Peter L.	Hazelton
Virginia	
Guilak, Hooshang	Charlottesville
Maddock, Robert K.	Norfolk
Washington	
Potts, John E.	Walla Walla
Wisconsin	
Lieberthal, Alan S.	Milwaukee
Other Countries	
Canada	
Hazlett, Barbara E. McK.	Toronto
New Zealand	
Beaven, Donald W.	Christchurch

NEWS OF AFFILIATE ASSOCIATIONS

The DIABETES ASSOCIATION OF ATLANTA held a meeting on December 10 at the Fulton County Medical Society Academy of Medicine. Guest speaker was Norman G. Anderson, M.D., Oak Ridge, Tennessee, whose subject was "Towards a Molecular Pathology."

The LOUISIANA DIABETES ASSOCIATION held its ninth annual one-day scientific session at the Bellemont Motor Hotel in Baton Rouge on November 7.

F. Gilbert McMahon, M.D., New Orleans, was the featured speaker. Joseph Dingman, M.D., was moderator during a panel discussion on "Oral Hypoglycemic Agents." Panelists were Douglas Gordon, M.D., D. W. Hayes, M.D., A. A. Herold, Jr., M.D., and Sol B. Stern, Jr., M.D.

NEWS NOTES

EIGHTH BAHAMAS MEDICAL CONFERENCE

The Eighth Bahamas Medical Conference was held from November 27 to December 17 at the British Colonial Hotel, Nassau, Bahamas. Speakers included:

Leonard L. Madison, M.D., Associate Professor of Medicine, University of Texas, Southwestern Medical School, Dallas, Texas: "The Mechanism of Action of the New Oral Hypoglycemic Agents (Sulfonylureas and DBI)" and "The Treatment of Choice (Insulin vs. Hypoglycemic Agents in the Treatment of Different Forms of Diabetes Mellitus)";

Roger Unger, M.D., Clinical Instructor in Medicine, University of Texas, Southwestern Medical School, Dallas: "Tests Used in the Diagnosis of Diabetes Mellitus, Including the Tolbutamide Response Test," and "The Precise Use of the Oral Hypoglycemic Agents in the Treatment of Diabetes Mellitus."

ADA MEMBERS TAKE PART IN POSTGRADUATE INSTITUTE

The Fourth Postgraduate Institute of The Potomac-Shenandoah Valley P G Institute, sponsored by The Eastern Panhandle Medical Society and The West Virginia Chapter of The American Academy of General Practice was held in Martinsburg, West Virginia, October 16-18. Members of the American Diabetes Association who took part in the program were: Perry S. MacNeal, M.D., Philadelphia, "Stay Alive"; Henry T. Ricketts, M.D., Chicago, "Present Status of the Oral Hypoglycemic Agents"; and Thomas H. McGavack, M.D., moderator of a panel discussion. The newest ADA scientific exhibit entitled "Diabetes—Today and Tomorrow: The Expanding Role of the Doctor," was displayed at the Institute.

PERSONALS

ROBERT S. BALDWIN, M.D., of Marshfield, Wisconsin, presented a discussion of "The Lente Insulins" at the annual meeting and scientific program of the Wisconsin Society of Internal Medicine, held at the Marshfield Clinic September 19.

ARTHUR A. HEROLD, SR., M.D., Shreveport, was presented a plaque in recognition of his work in the field of diabetes by the Louisiana Diabetes Association on November 7 in Baton Rouge. The plaque was presented by F. W. Pickell, M.D., president of the Affiliate. Dr. Herold, who was graduated from Tulane University Medical School in 1907, founded the North Louisiana Sanitarium and was its president and manager for several years. Formerly president of the Louisiana Diabetes Association and governor for the State of Louisiana of the American Diabetes Association, Dr. Herold is councilor for the Southern Medical Association and a life member of the American College of Physicians. He has served as a State delegate to the American Medical Association for several years.

NECROLOGY

IRMGARD DRESEL, Far Hills, New Jersey, born May 5, 1896.

THEODORE R. FAILMEZGER, Madison, New Jersey, born August 20, 1904.

ADOLPH GRUNBERG, Torquay, Devon, England, born January 30, 1908.

HORACE D. HINSHAW, La Porte, Indiana, born December 12, 1928.

EMMET T. SCALES, Des Moines, Iowa, born February 4, 1901.

SAMUEL SILBERT, New York, New York, born January 24, 1894.

B. C. WALLACE, Waxahachie, Texas, born August 31, 1909.

